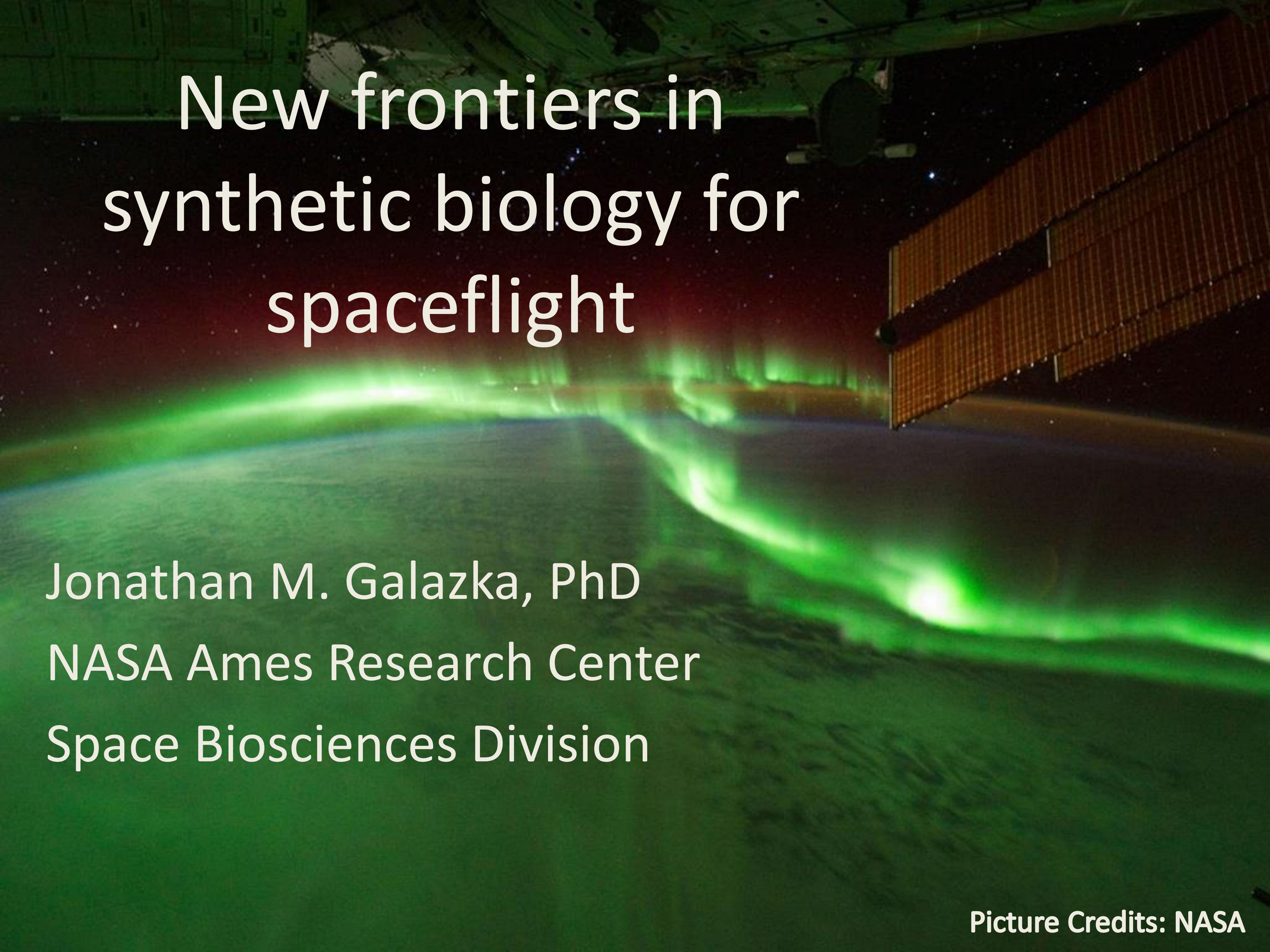
New frontiers in synthetic biology for spaceflight

Jonathan M. Galazka, PhD
NASA Ames Research Center
Space Biosciences Division



Picture Credits: NASA

Funding

Funding
NASA Space Technology Mission Directorate Center Innovation Fund
NASA Advanced Exploration Systems

Team

Dr. John A. Hogan (NASA)
Dr. Asif Rahman (U. of New Mexico)
Dr. Aditya Hindupur (Wyle Labs)

Dr. Michael Dougherty (Wyle Labs)
Natalie Ball (Wyle Labs)
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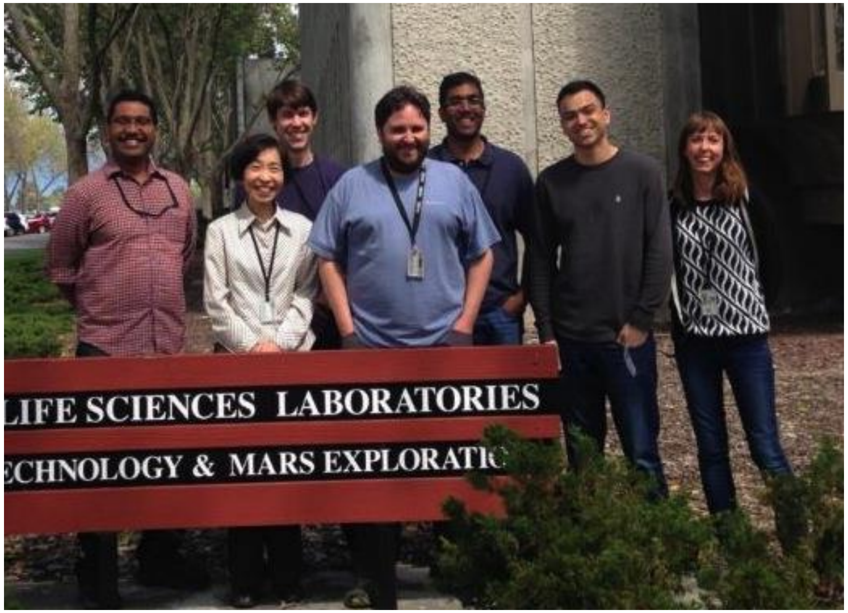


NASA Ames Team Members

Dr. John Hogan

Samantha Fleury & Lily Neff

WASHU Collaborators

Fuzhong Zhang
Chris Bowen
Cameron Sargent
Sarah Rommelfanger

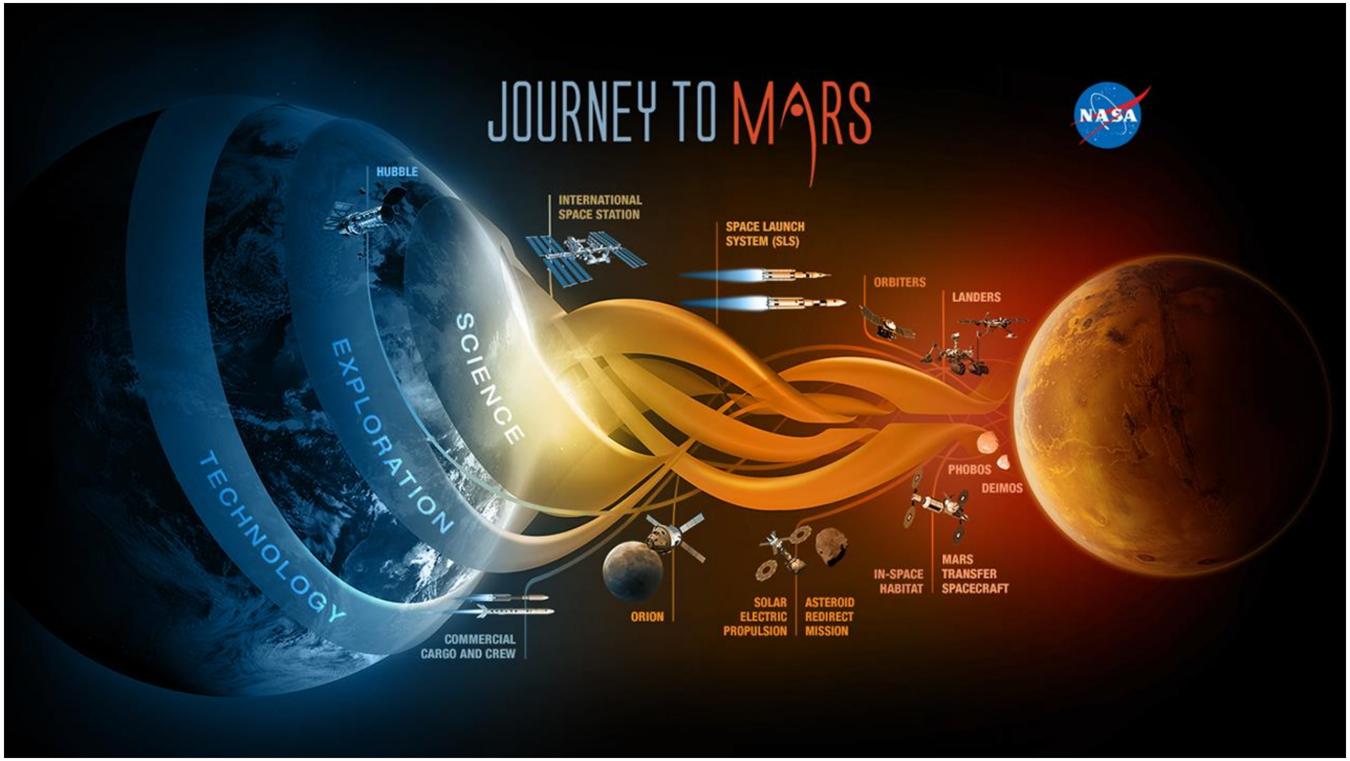
Moving to Deep Space

Challenges…

Increased radiation exposure risk

Limited opportunity for crew return (launch window occurs every 25 months)

Limited opportunity for resupply

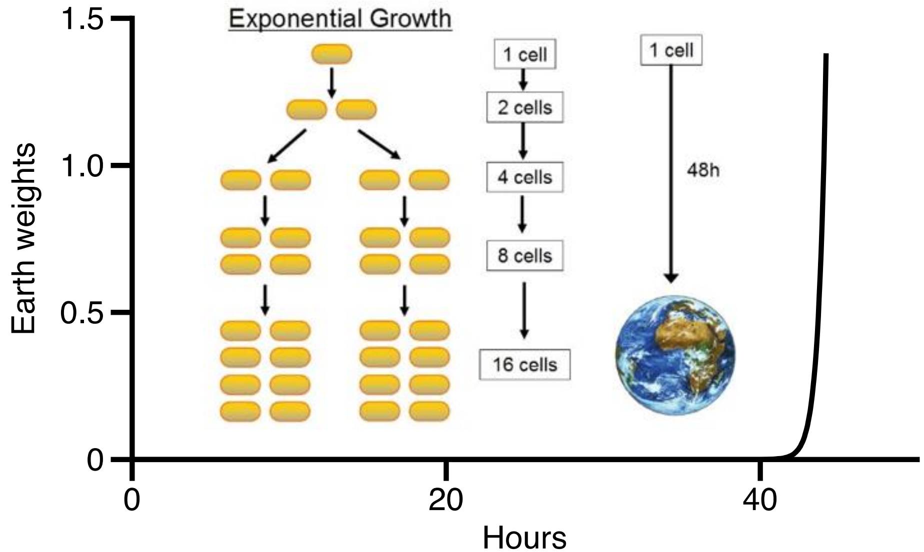


Missions to Deep Space are enabled by robust technologies that:

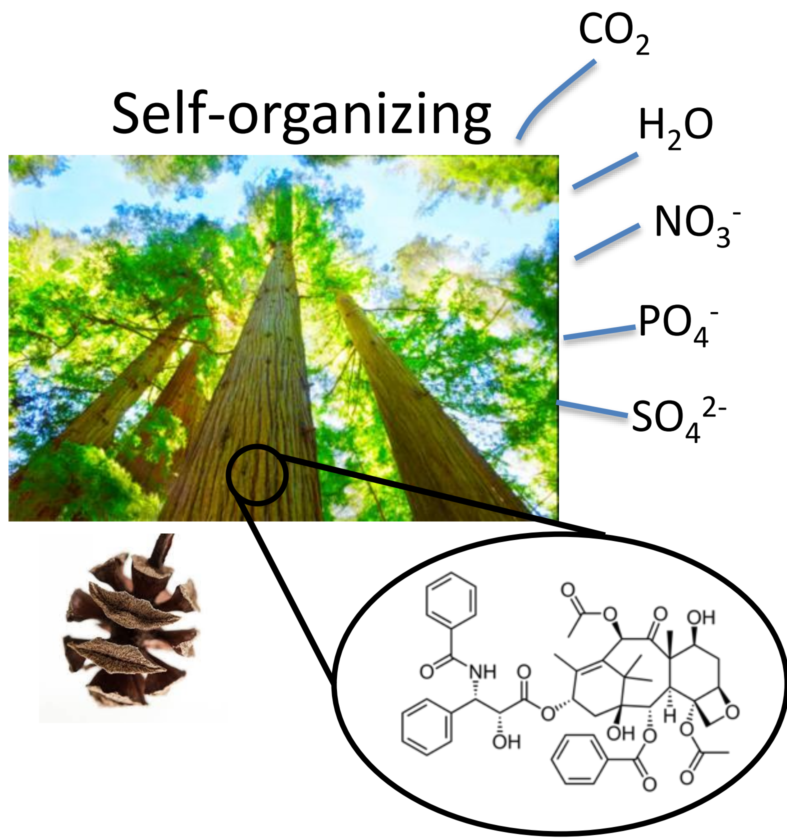
- Make crew more self-sufficient
- Allow utilization of local resources

Potential of Microbial Manufacturing in Space

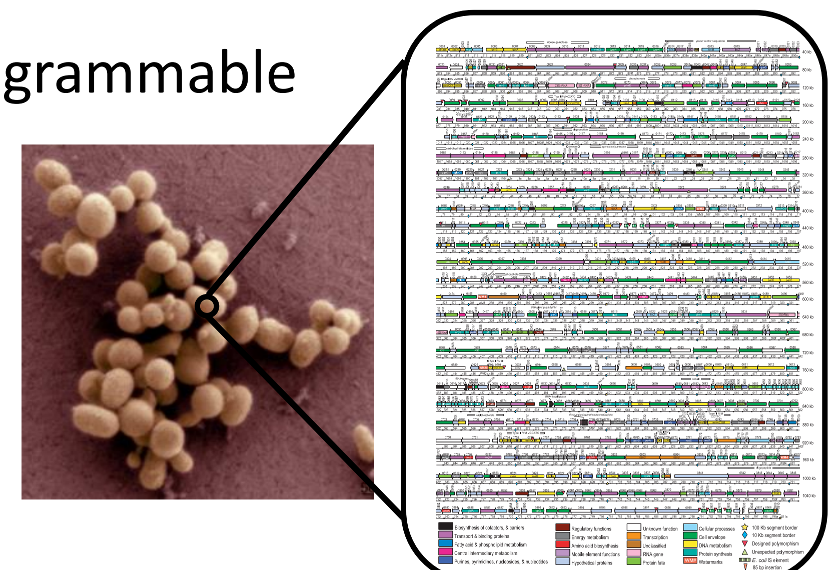
Scalable



Self-organizing



Programmable



Potential of Microbial Manufacturing in Space

Biological systems are:

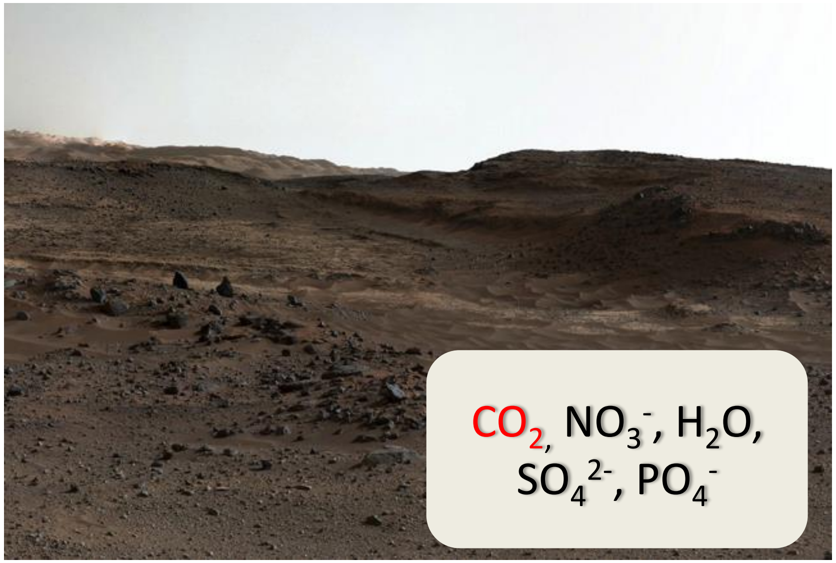
- Scalable
- Programmable
- Precise (pure isomers)
- The only route of production in some cases (protein therapeutics)
- Low T° and pressure
- Regenerable

Credits: NASA

Barriers to Microbial Manufacturing in Space

Space technologies must be…

- Robust
- Simple to operate
- Stable during storage
- Compatible with available resources and infrastructure



Mount Sharp, Mars. Credits: NASA.

We will have this

Not this

YPD:

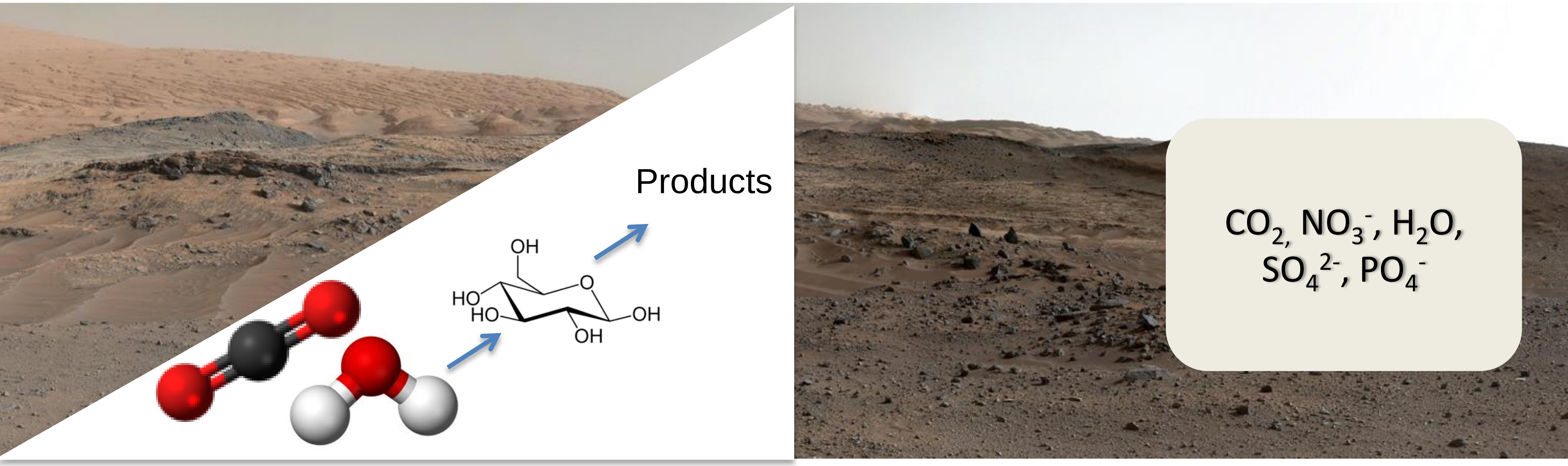
0.3% yeast extract

1% peptone

1% glucose

Material conversion

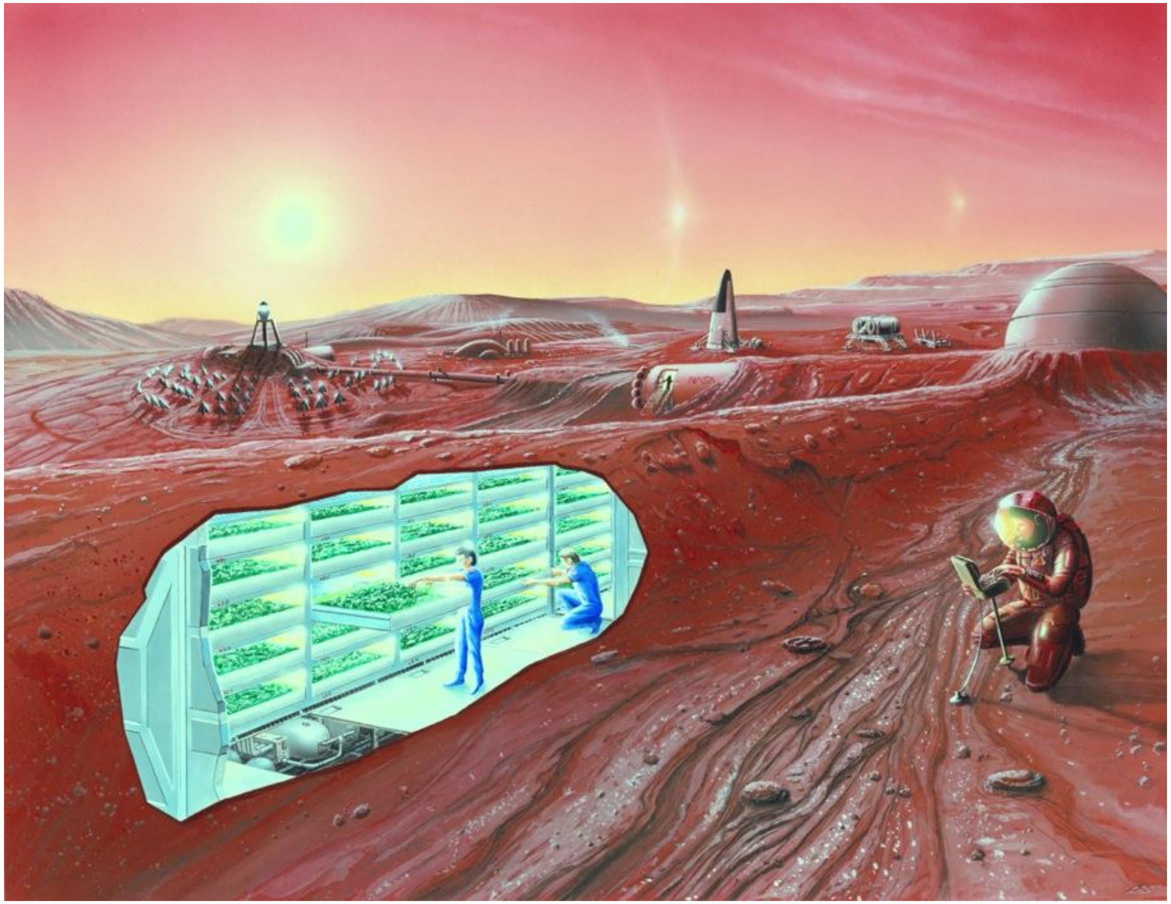
Iowa, Earth



Mount Sharp, Mars

Photoautotrophs

$$6CO_2 + 6H_2O \dashrightarrow C_6H_{12}O_6 + 6O_2$$

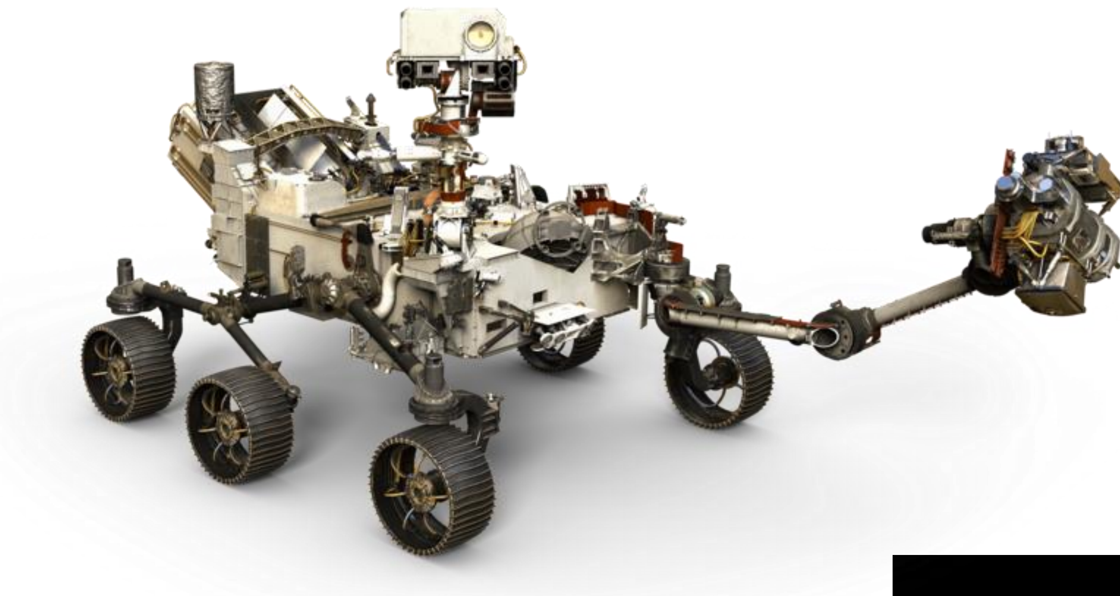


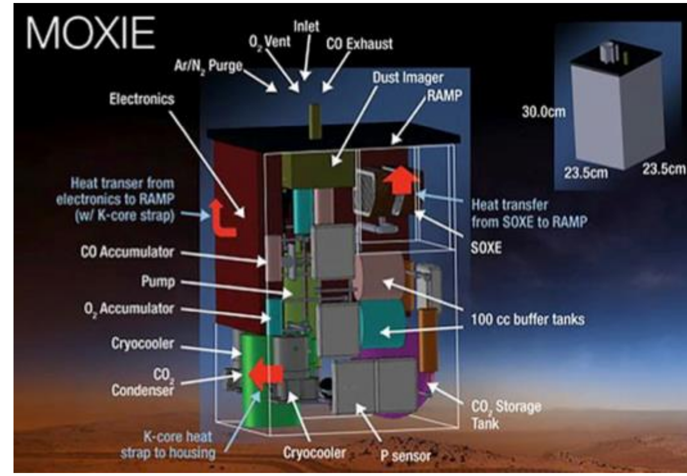
Credits: NASA

But…

- Usually have slower growth rates than heterotrophs
- Require photobioreactors

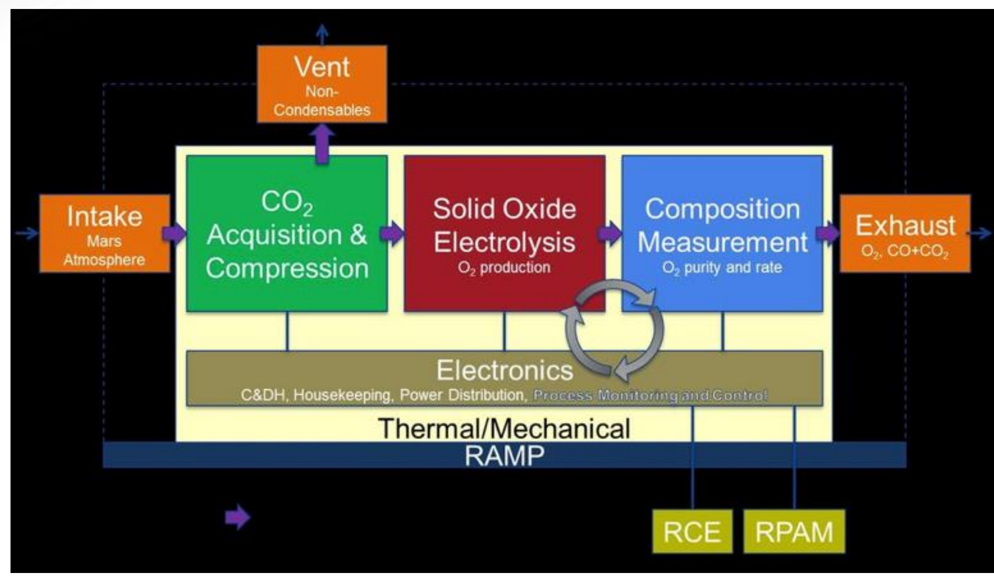
MOXIE: Mars Oxygen ISRU Experiment





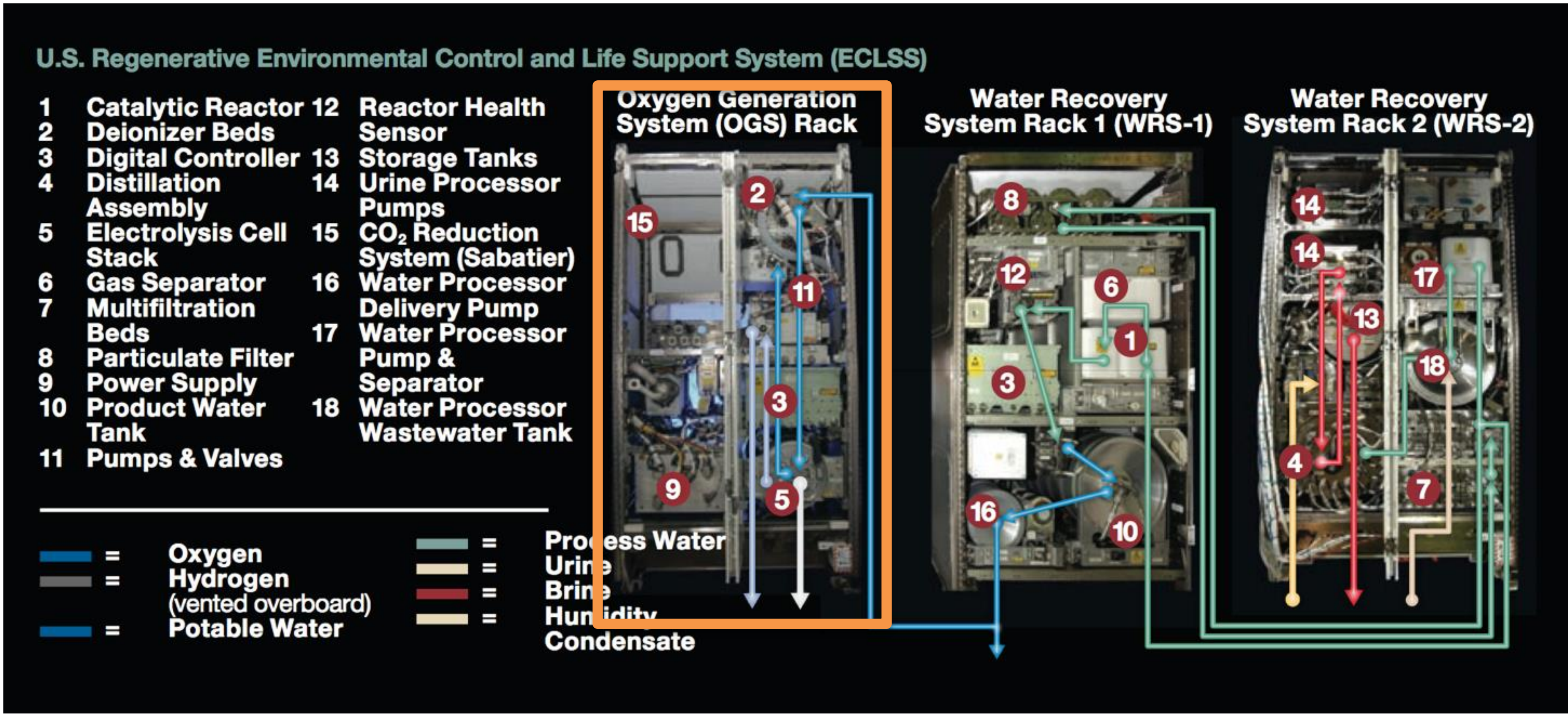
$2CO_2$ --> $2CO + O_2$

Clostridium spp.



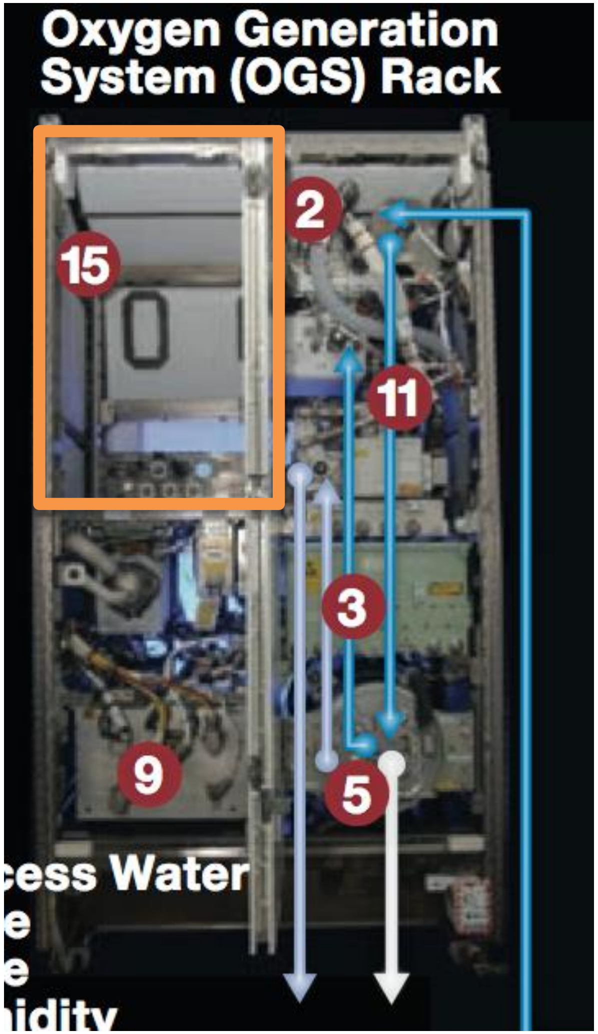
Picture Credits: NASA

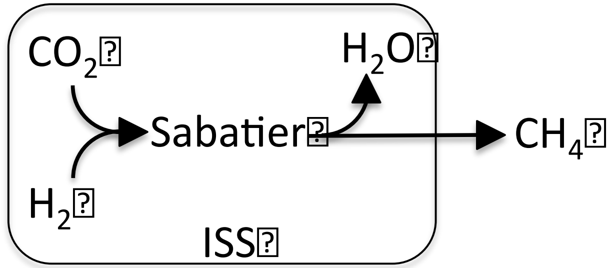
Environmental Control and Life Support System



Credits: NASA
“Reference Guide to the International Space Station”

Methane production by Sabatier





Astronaut Doug Wheelock installing Sabatier system on ISS

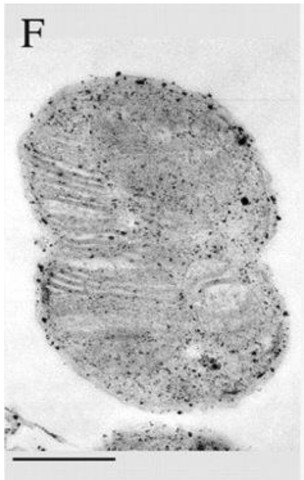
- Reacts CO_2 and H_2 to form CH_4 and H_2O
- H_2O is recycled
- CH_4 vented to space
- Operational since 2010

"Waste" CH_4 could be fed to methanotrophs in a microbial manufacturing scheme.

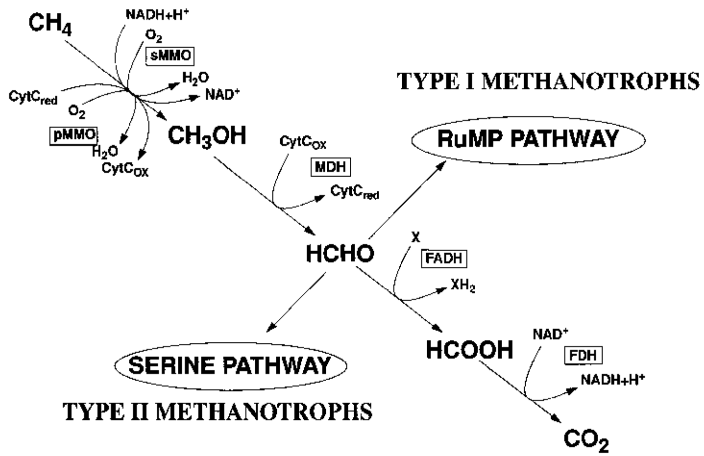
Picture Credits: NASA

Why Not Bacterial Methanotrophs?

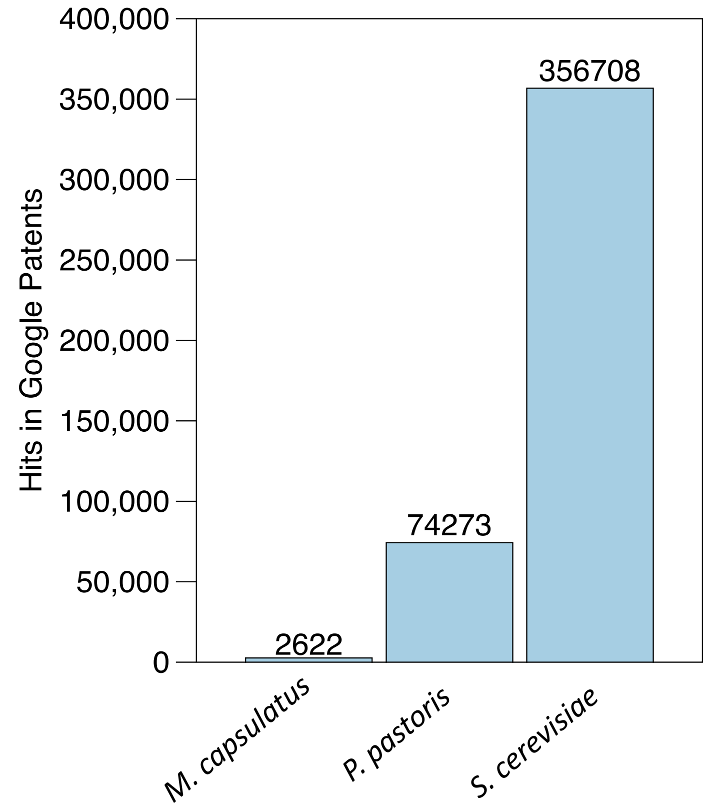
M. capsulatus



J. Bacteriol. October 2003 vol. 185 no. 19 5755-5764

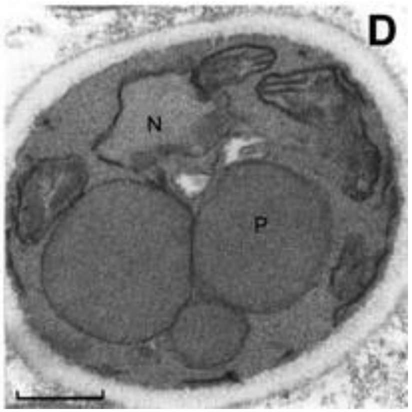


Microbiol. Mol. Biol. Rev. June 1996 vol. 60 no. 2 439-471



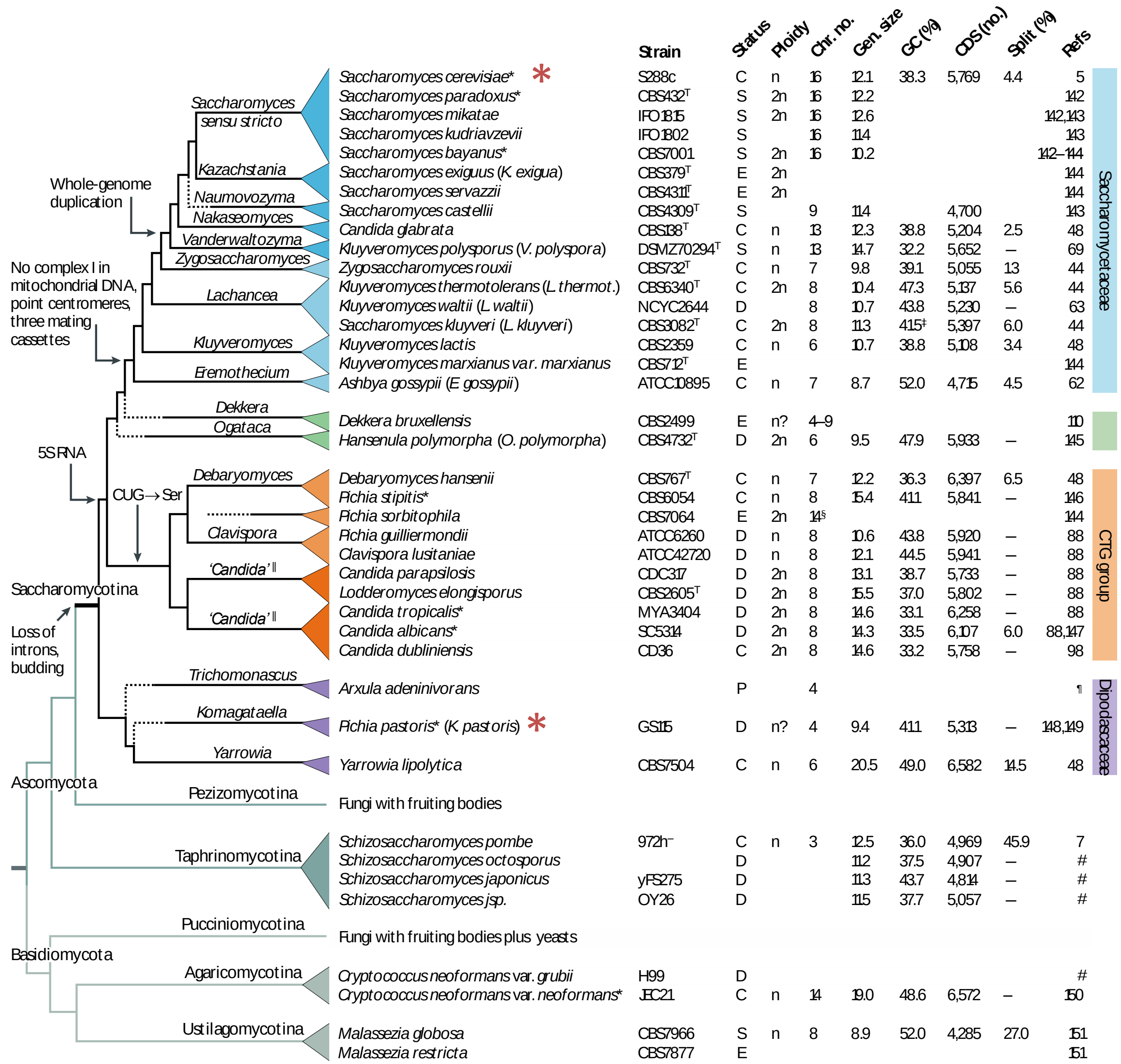
- NASA STTR to Mango Materials (Small Technology Transfer Innovation Research)
- *"A Novel, Membrane-Based Bioreactor Design to Enable a Closed-Loop System on Earth and Beyond"*
- *"…a membrane bioreactor system to produce a biopolymer from methane gas…will enable bacterial growth and biopolymer production to occur in microgravity environments…"*

Pichia pastoris (Komagataella phaffii)



Ida J. van der Klei et al. EMBO J. 1998;17:3608-3618

- Diverged from *S. cerevisiae* ~200 MYA
- Methylotrophic yeast
- Widely used as a protein production host
- Capable of producing reduced and glycosylated proteins
- Grows to very high cell density (optical densities up to 630, Dr. Julia Cino, New Brunswick Scientific)



Dujon, 2010, Nature Reviews Genetics

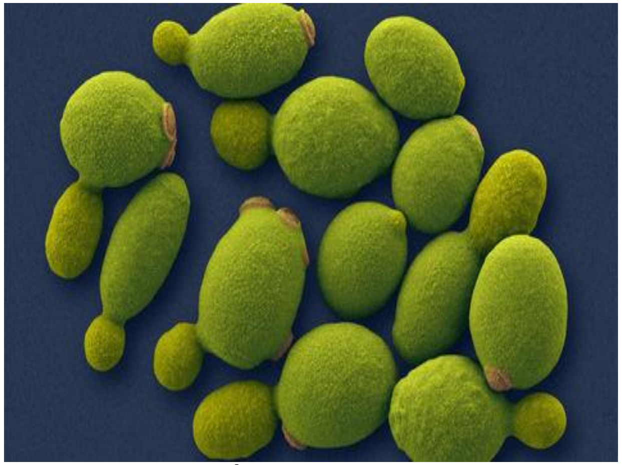
Why *Pichia pastoris*?

1. Established synthetic biology platform

   Used to produce Trypsin, murine TNFα, and FDA approved drugs Kalbitor (60 amino acid peptide to treat hereditary angioedema) and Jetrea (proteolytic enzyme to treat symptomatic vitreomacular adhesion)

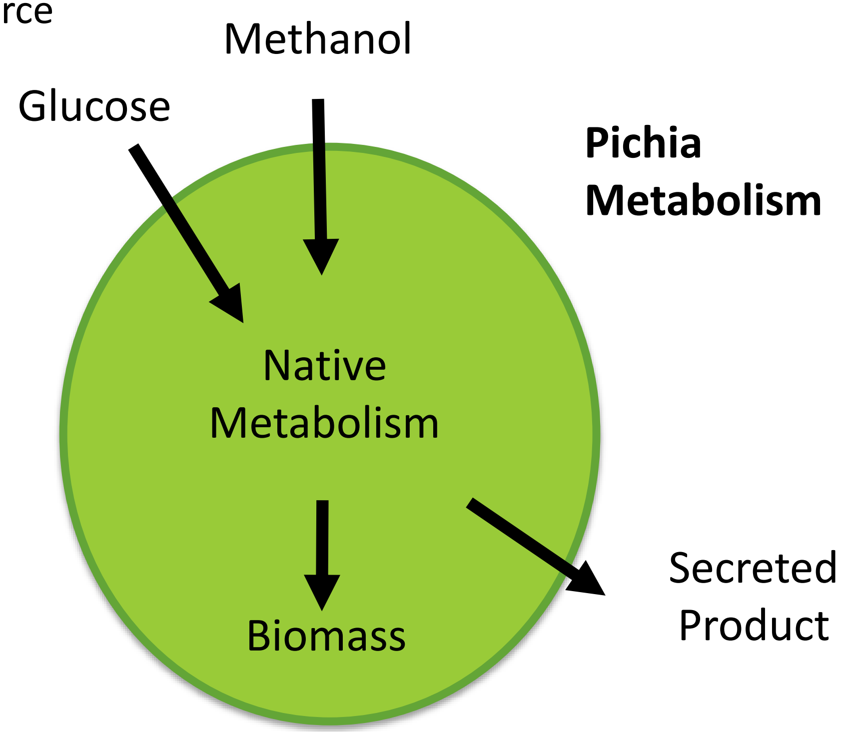
2. Methylotrophic yeast

   Can utilize methanol (CH_3OH) as a carbon source

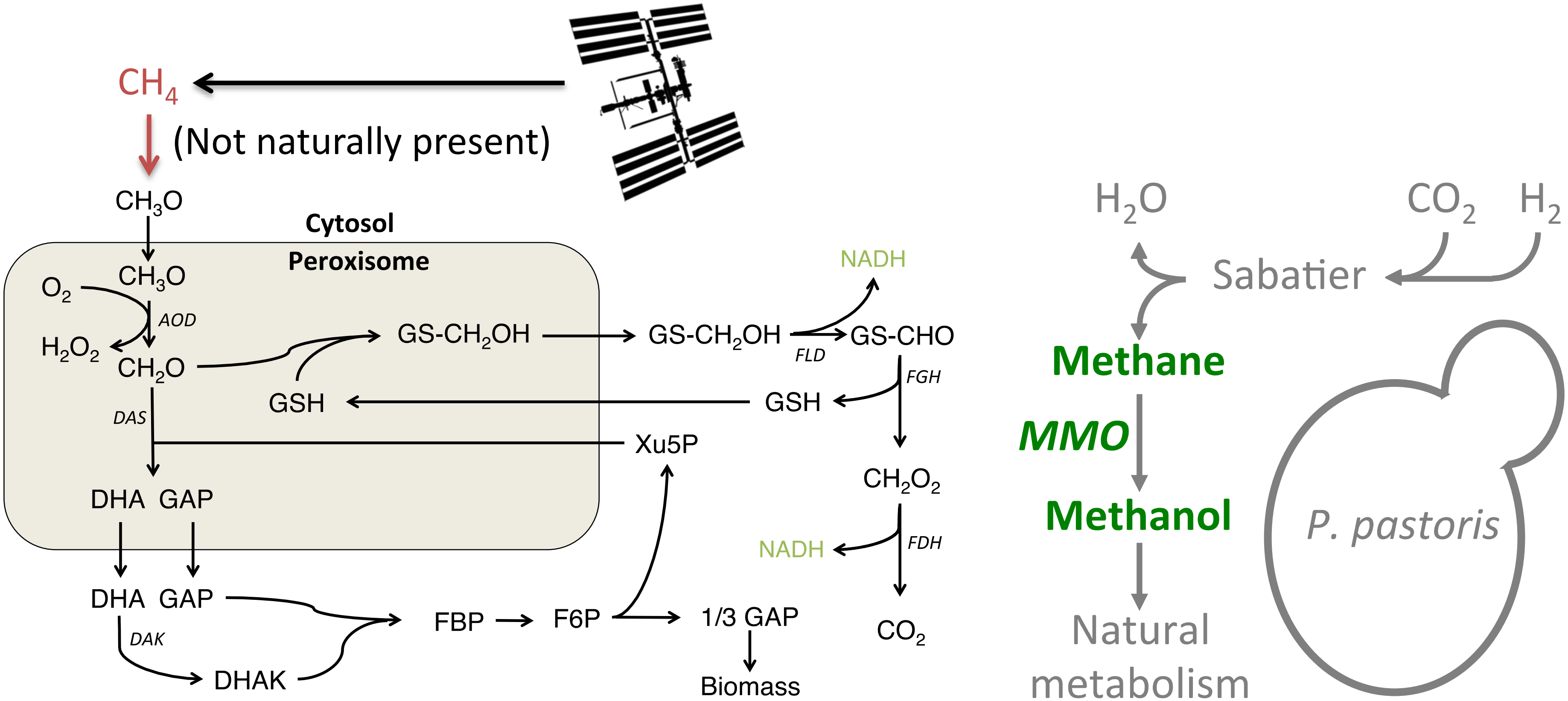


Pichia pastoris

Dennis Kunkel Microscopy, 2017, Science

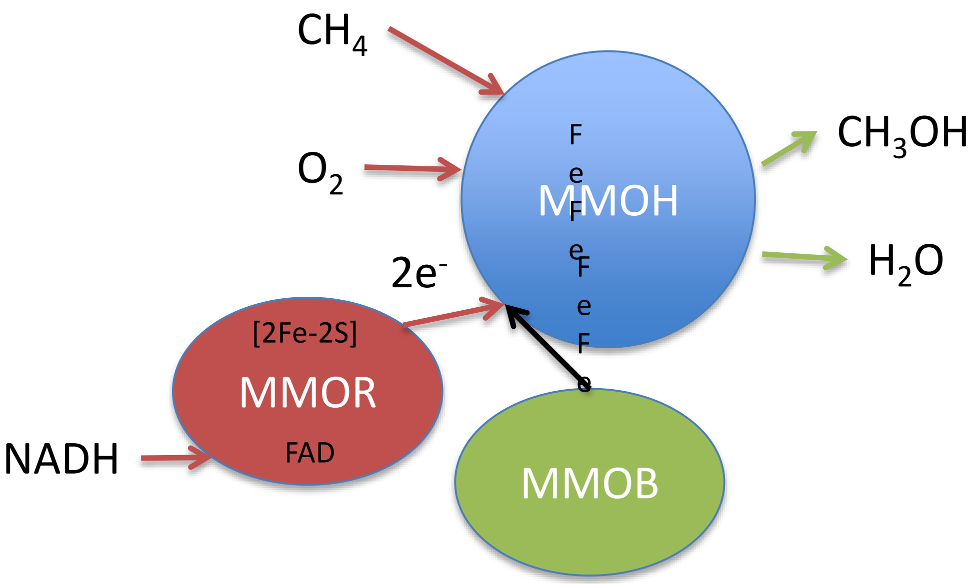


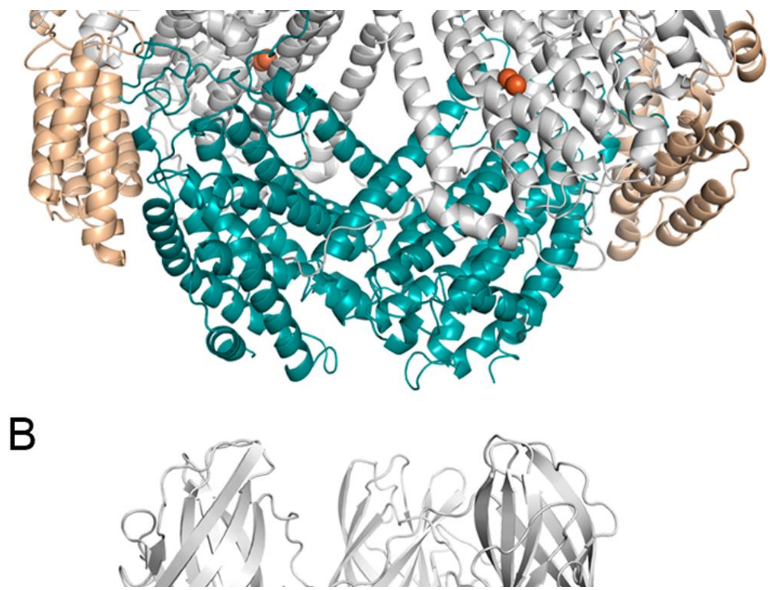
Porting methane metabolism to *P. pastoris*



Methanol (CH_3O) is oxidized to formaldehyde (CH_2O) by alcohol oxidase (*AOD*). Formaldehyde can either be oxidized to CO_2 through the successive action of formaldehyde dehydrogenase (FLD), *S*-formylglutathione hydrolase (*FGH*), and formate dehydrogenase (*FDH*), or appended to xylulose-5-phosphate and assimilated into biomass through a pathway involving dihyroxyacetone synthase (*DAS*) and dihydroxyacetone kinase (*DAK*).

Porting Soluble Methane Monooxygenase to *Pichia*





Sirajuddin and Rosenzweig. 2015.

1. Hydroxylase: MMOH
    1. alpha
    2. beta
    3. gamma

    Oxidizes methane hydroxylates methane to methanol

2. Reductase: MMOR

    Oxidizes NADH and transfers electrons to MMOH

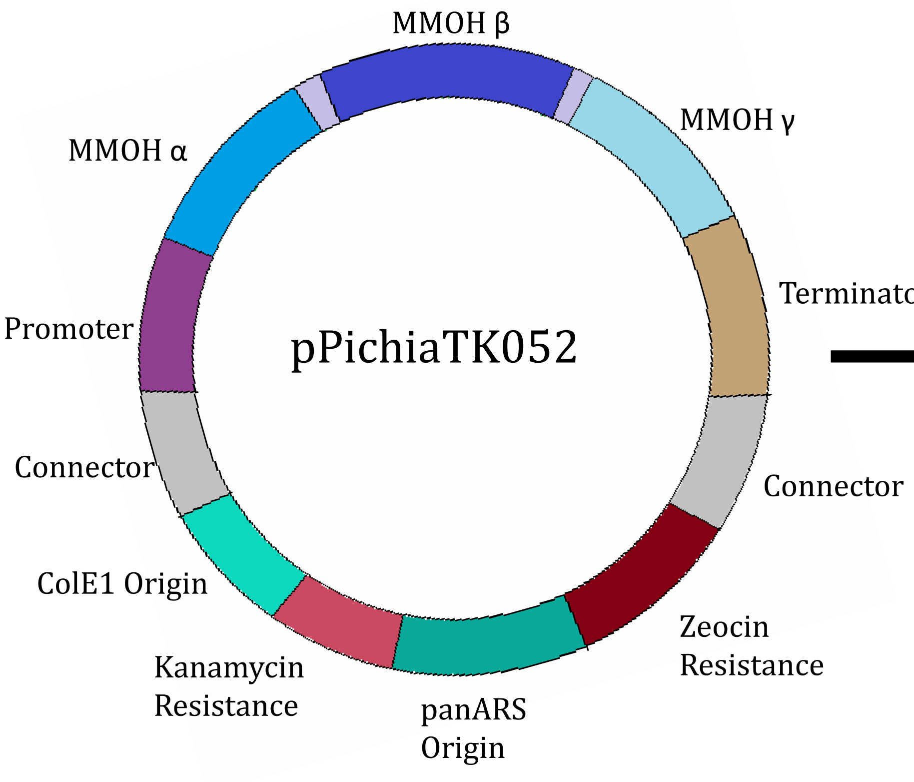
1. Regulatory: MMOB

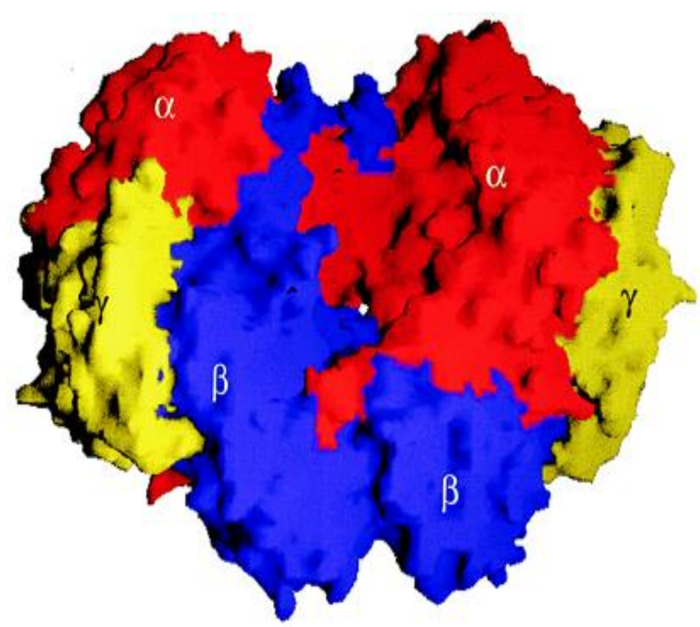
    Binds to same site as MMOR. May help drive cycle.

2. Assembly chaperone: MMOG

    Could help in assembly of MMOH

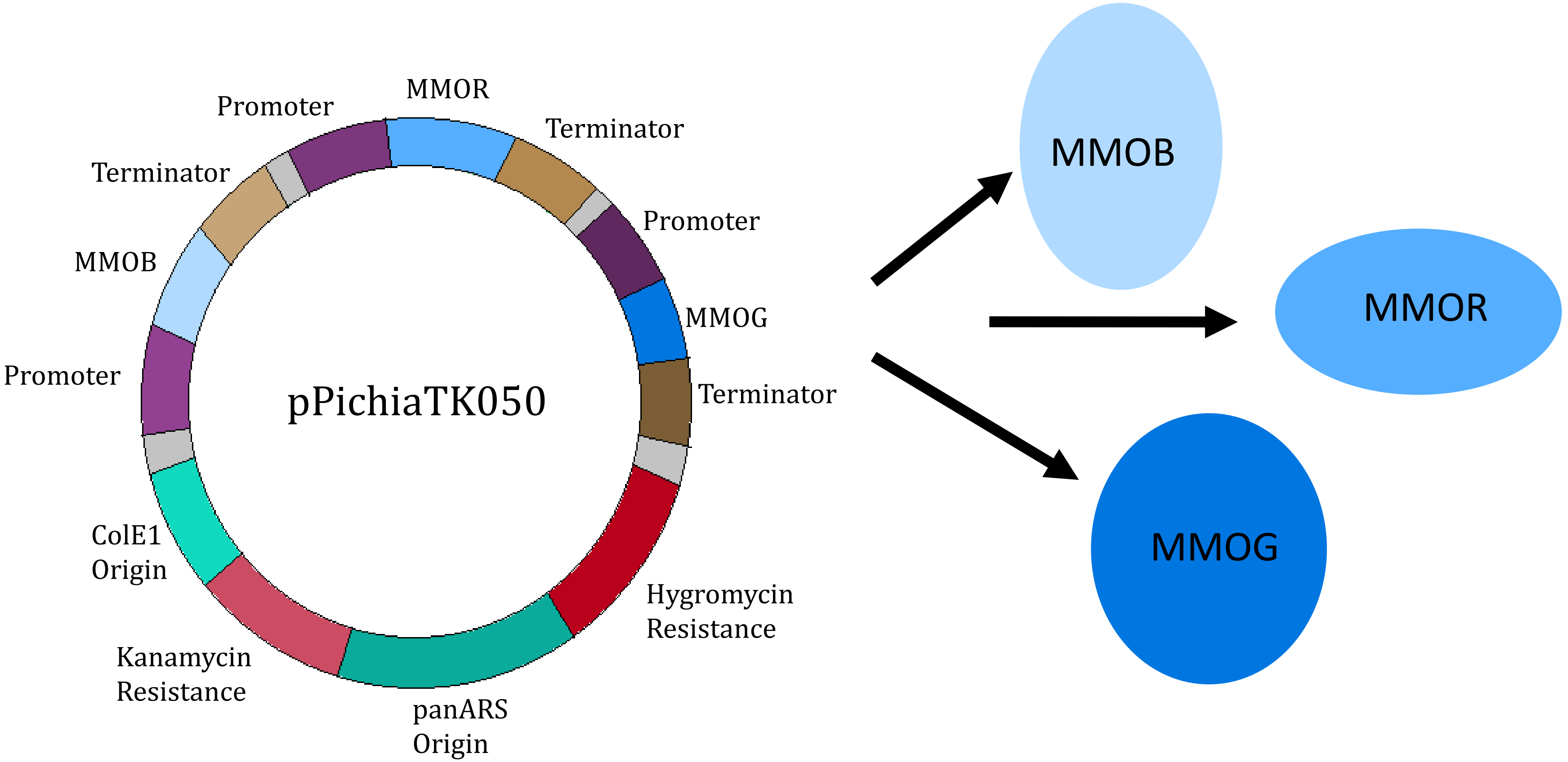
Plasmid I: Single transcript expression of MMOH





MMOH

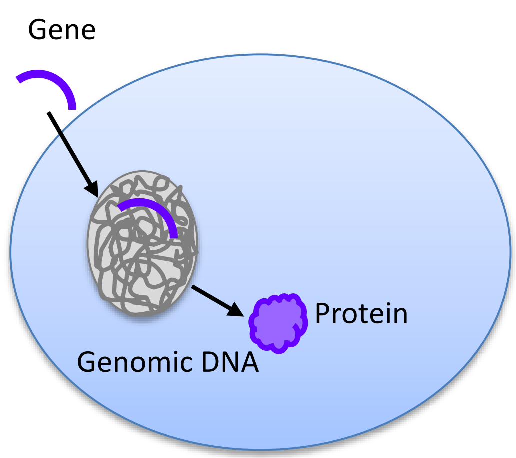
Plasmid II: Expression of accessory proteins



Introducing MMO genes to *Pichia*

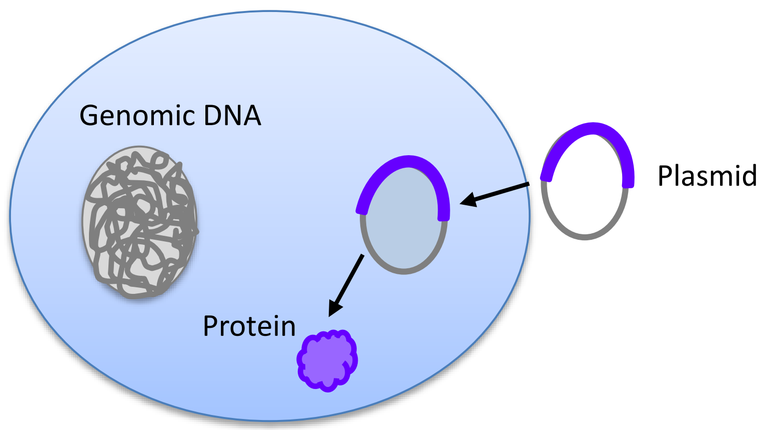
Genome Editing

- Insert gene into organismal genome
- CRISPR
- Homologous Recombination



Plasmid Transformation

- Gene inserted as a small independently replicating circle of DNA



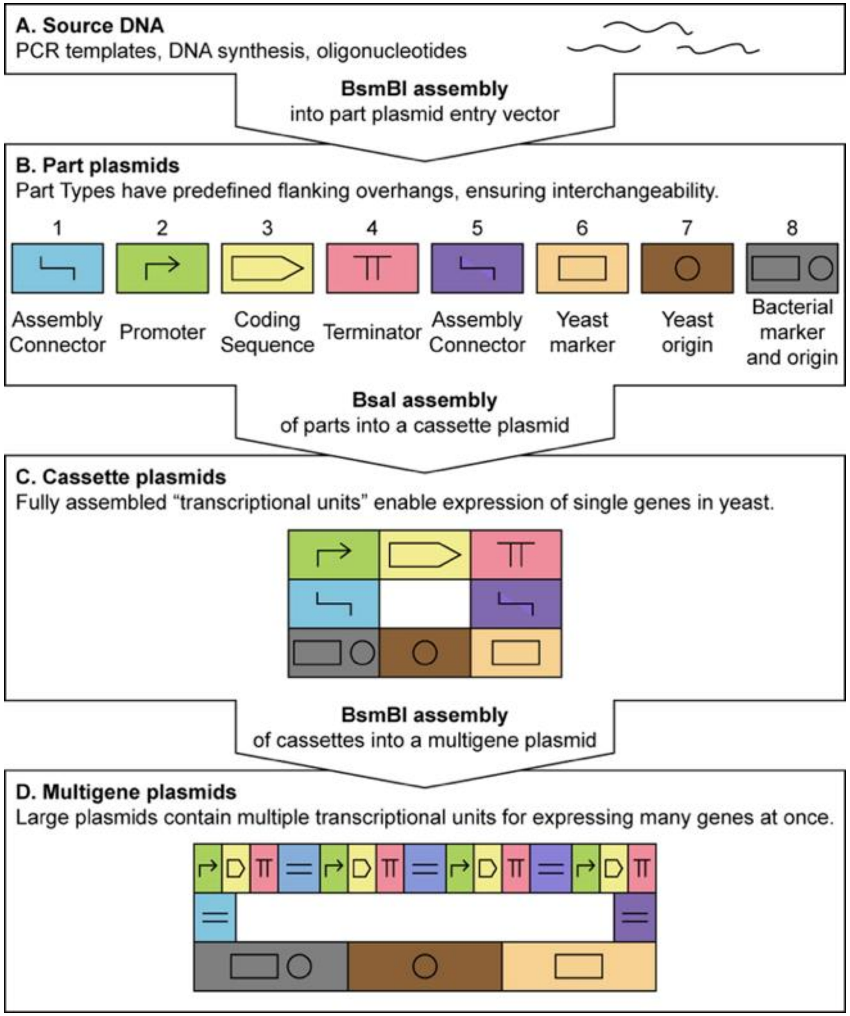
Developing *Pichia* tools

Pichia tools are limited compared to *S. cerevisiae*

Plasmid systems have only recently been developed

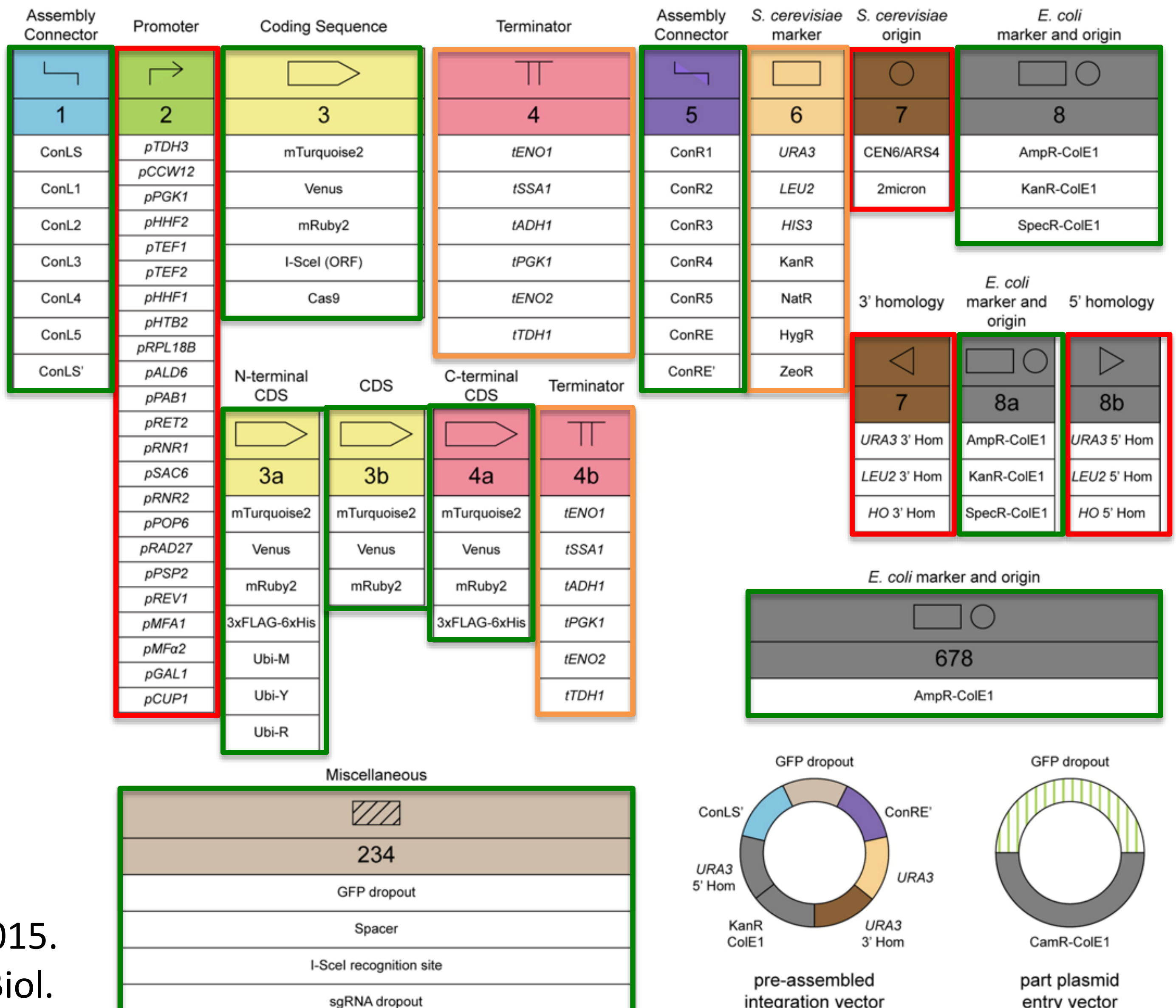
John Dueber at Berkeley has developed a "*S. cerevisiae* Toolkit"

This could be adapted for use in *Pichia*



Lee et al. 2015. ACS Synth Biol.

Building a *Pichia* toolkit



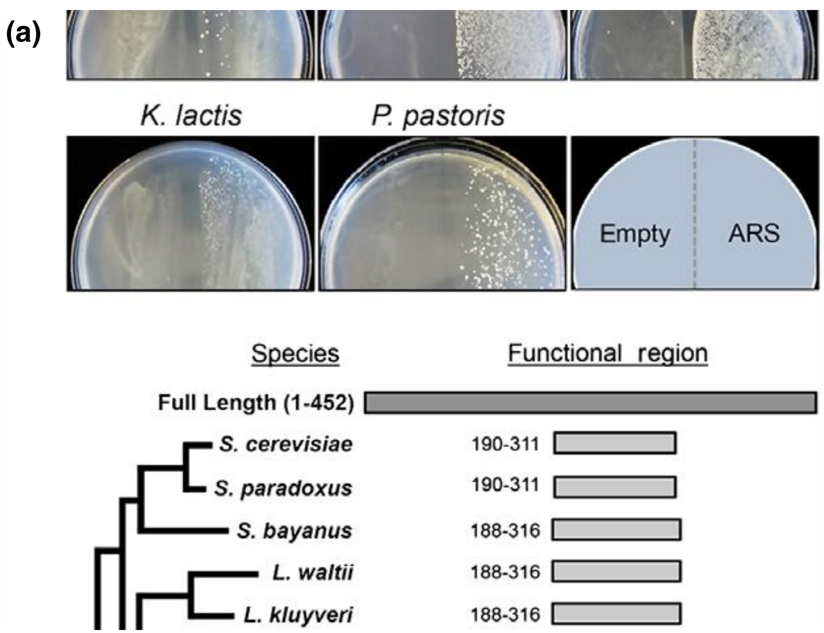
Lee et al. 2015.
ACS Synth Biol.

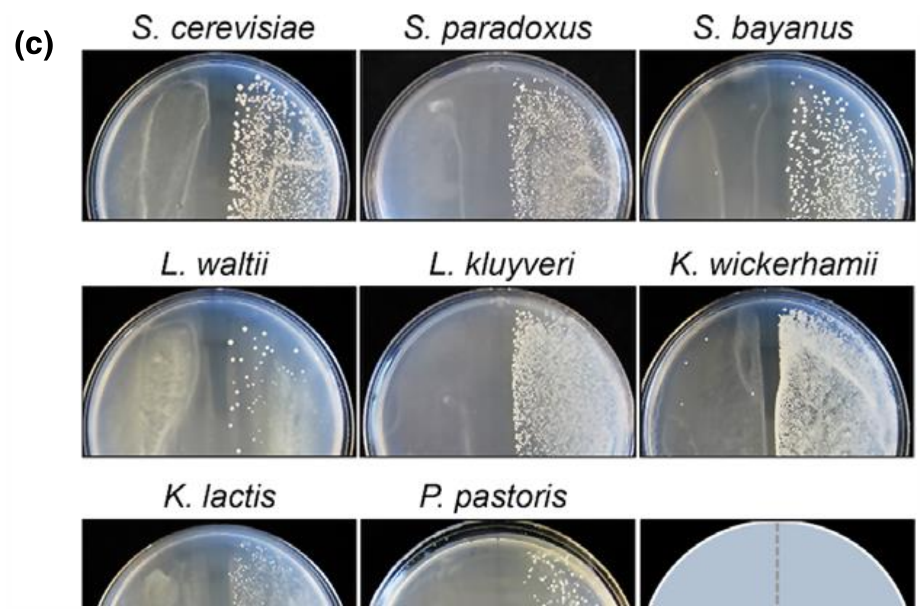
Origin of replication: panARS

An autonomously replicating sequence for use in a wide range of budding yeasts

Ivan Liachko & Maitreya J. Dunham

Department of Genome Sciences, University of Washington, Seattle, WA, USA



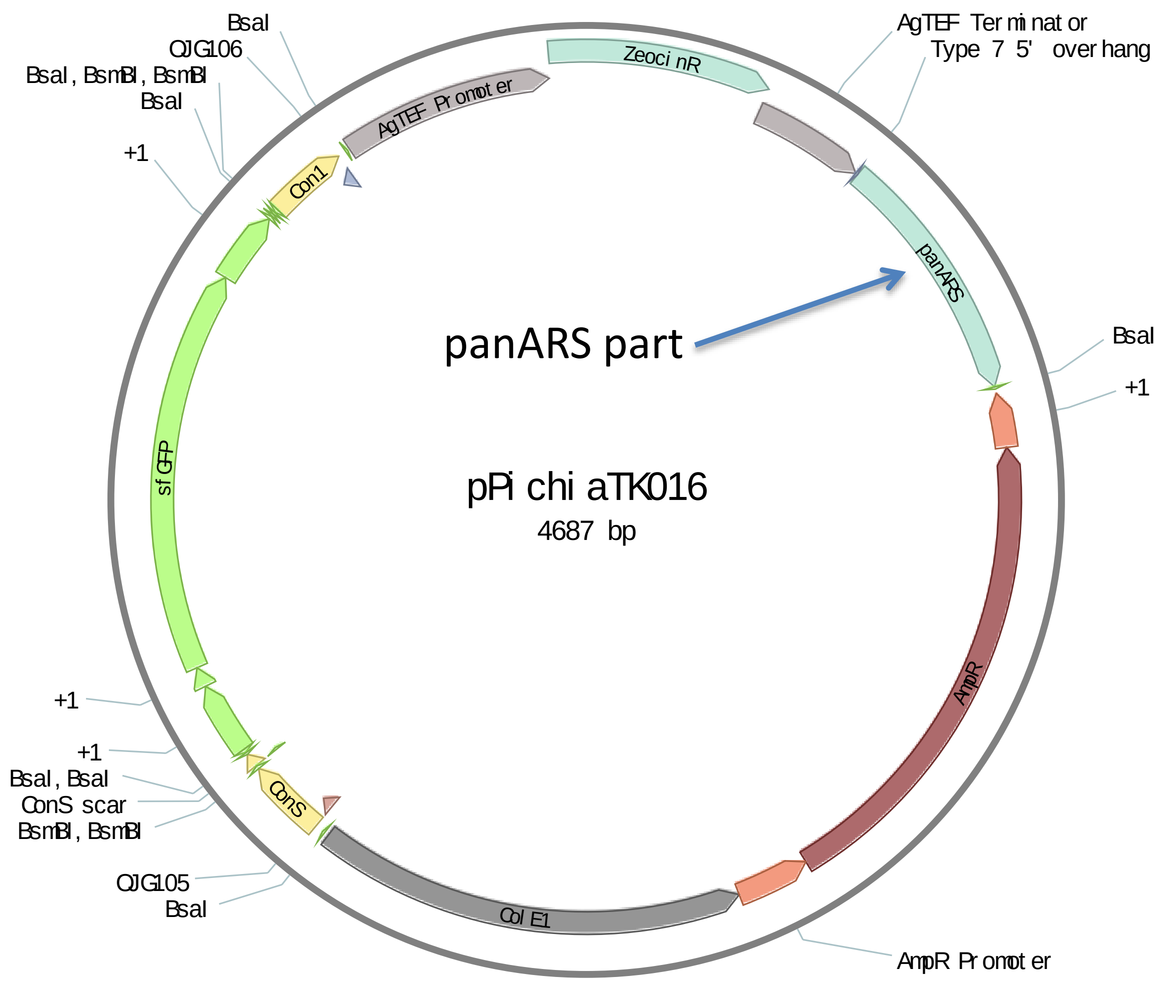


Plasmid system for *Pichia*

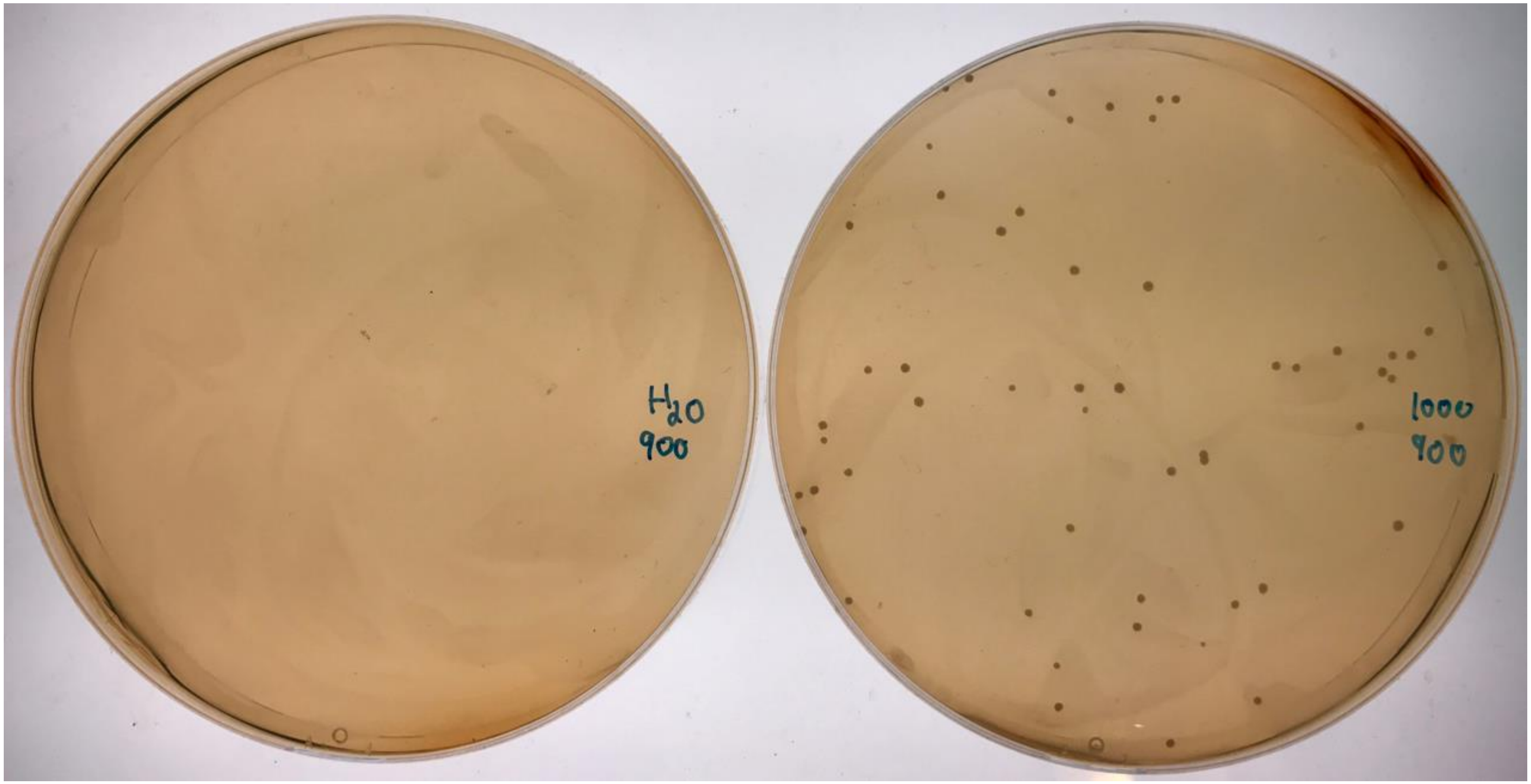
Adapted plasmid system from John Dueber by incorporating recently identified "panARS" sequence to allow replication of plasmid in *Pichia*.

Most other parts should be re-usable.

Constructed empty vector that should impart resistance to Zeocin.



Plasmid system for *Pichia*

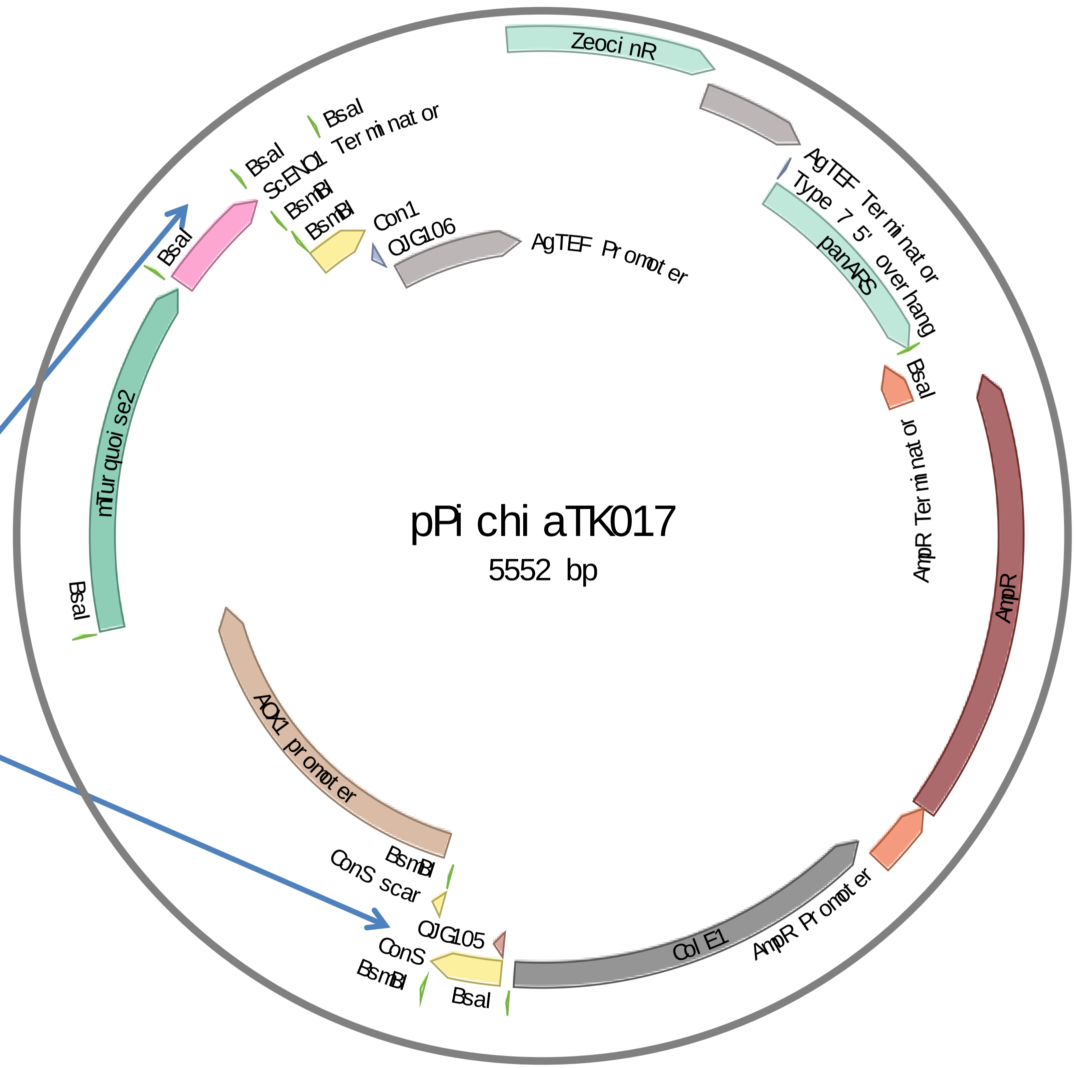


Water

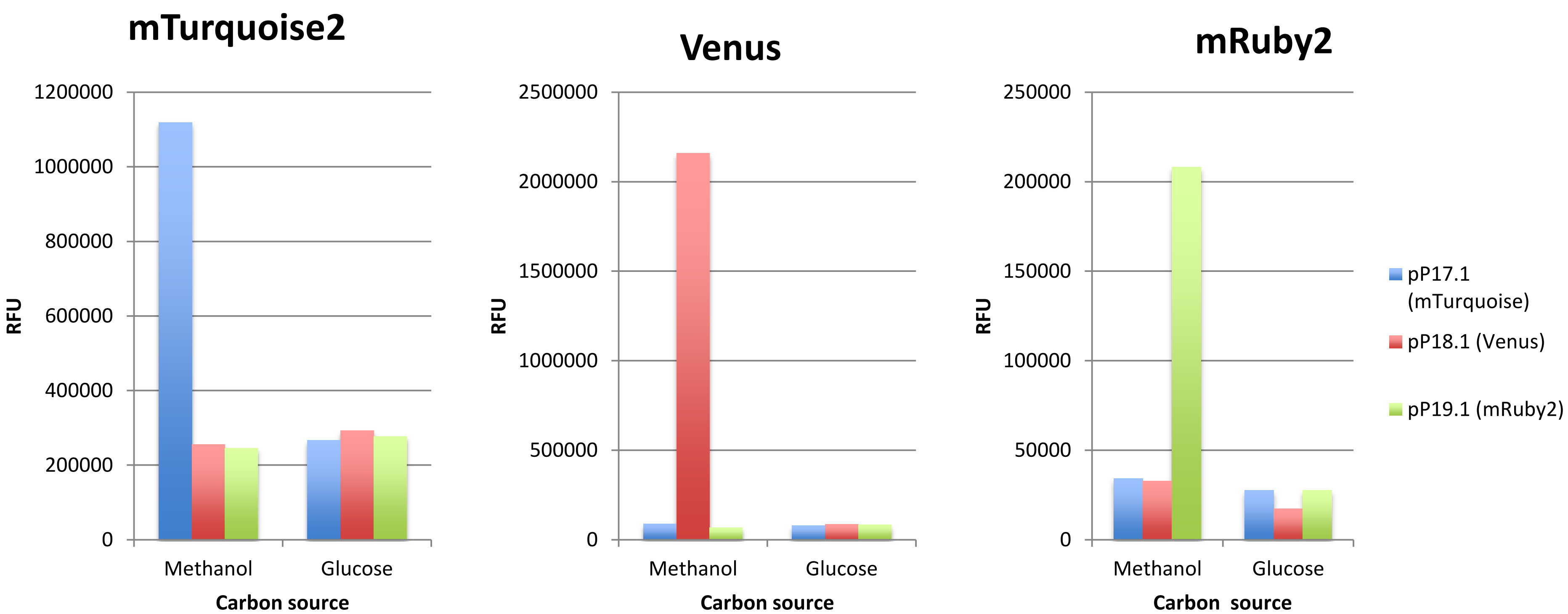
1 µg pTK16

Plasmid demonstration in *P. pastoris*

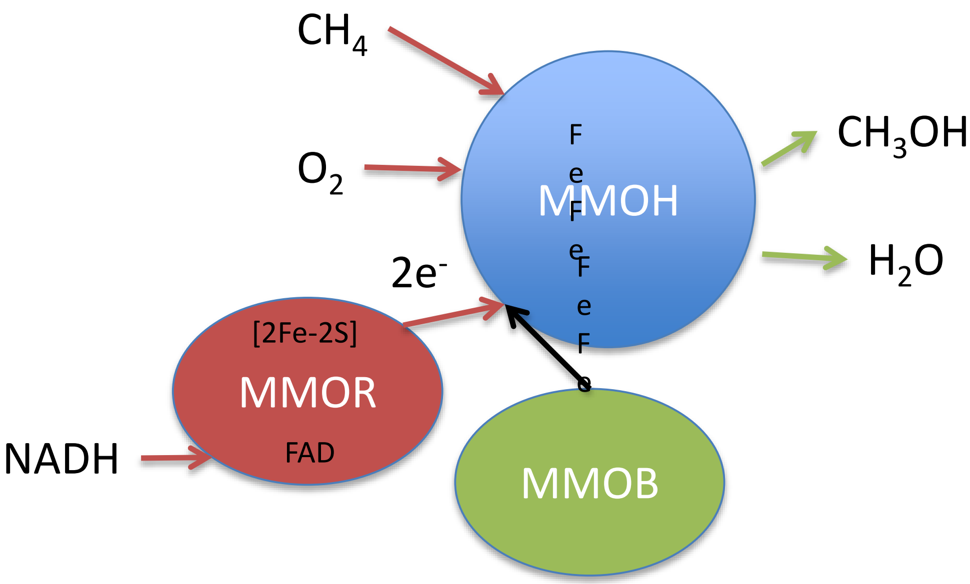
pPichia17 – AOX1p-mTurquoise-ScENO1
pPichia18 – AOX1p-Venus-ScENO1
pPichia19 – AOX1p-mRuby-ScENO1

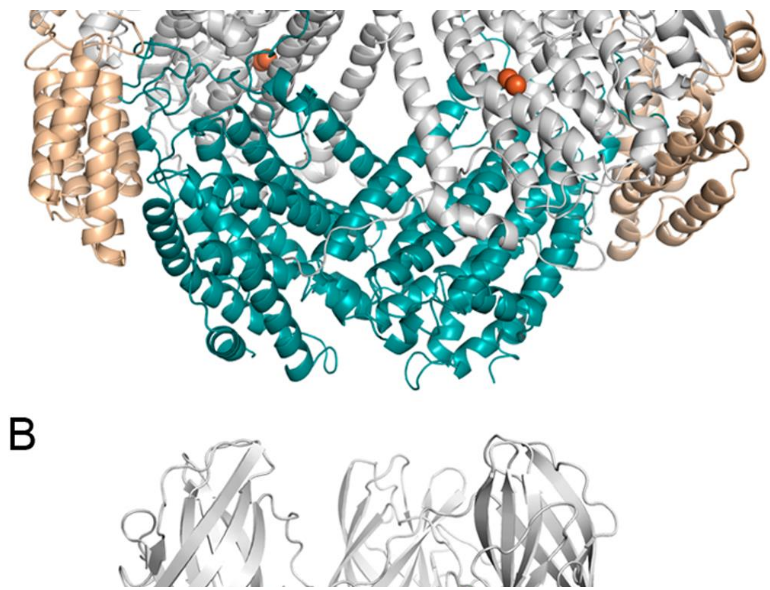


Plasmid demonstration in *P. pastoris*



Porting Soluble Methane Monooxygenase to *Pichia*





Sirajuddin and Rosenzweig. 2015.

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   Oxidizes methane hydroxylates methane to methanol

2. Reductase: MMOR

   Oxidizes NADH and transfers electrons to MMOH

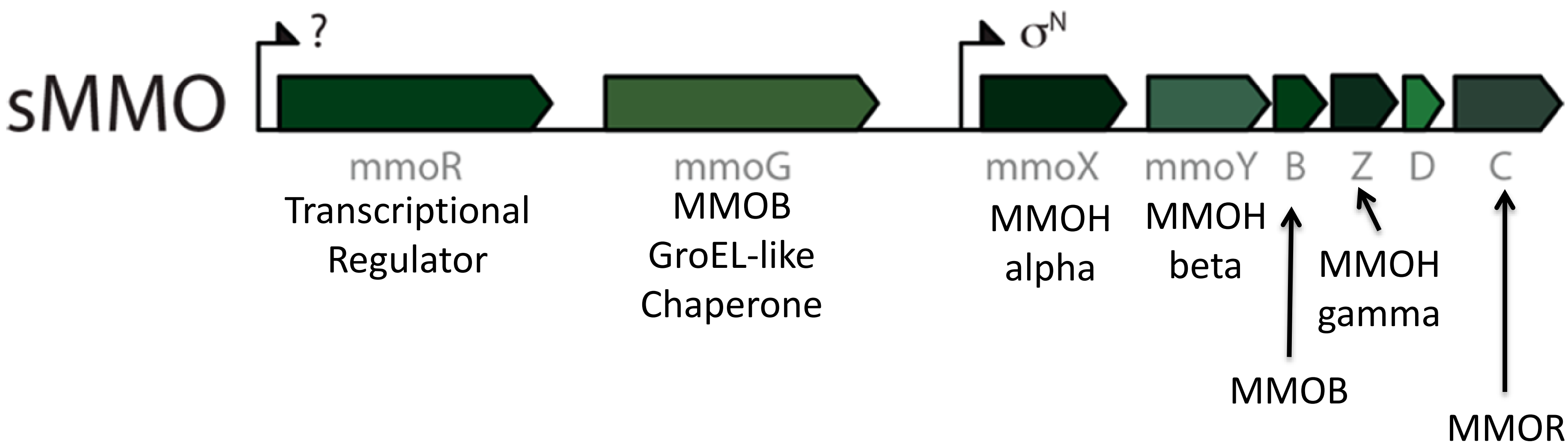
1. Regulatory: MMOB

   Binds to same site as MMOR. May help drive cycle.

2. Assembly chaperone: MMOG

   Could help in assembly of MMOH

MMO components are expressed as an operon



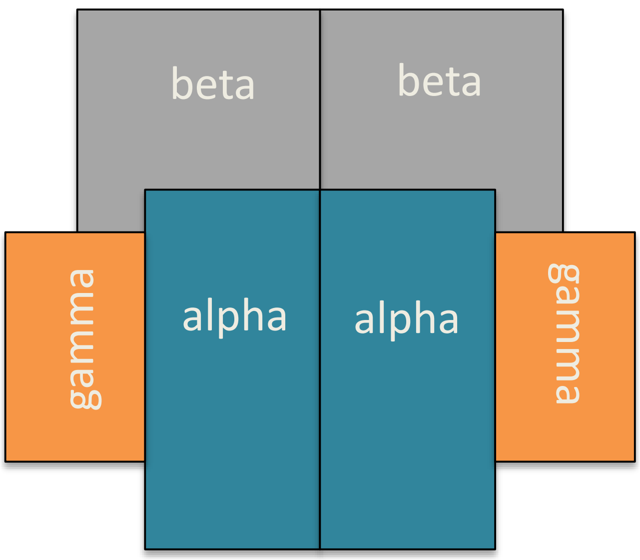
- Most studies are sMMO from *Methylococcus capsulatus* (Bath) or *Methylosinus trichosporium* OB3b

- MMO from *M. trichosporium* OB3b has a higher turnover number (3.5 vs. 0.2 – 1.0 s^{-1})

Sirajuddin and Rosenzweig. 2015.

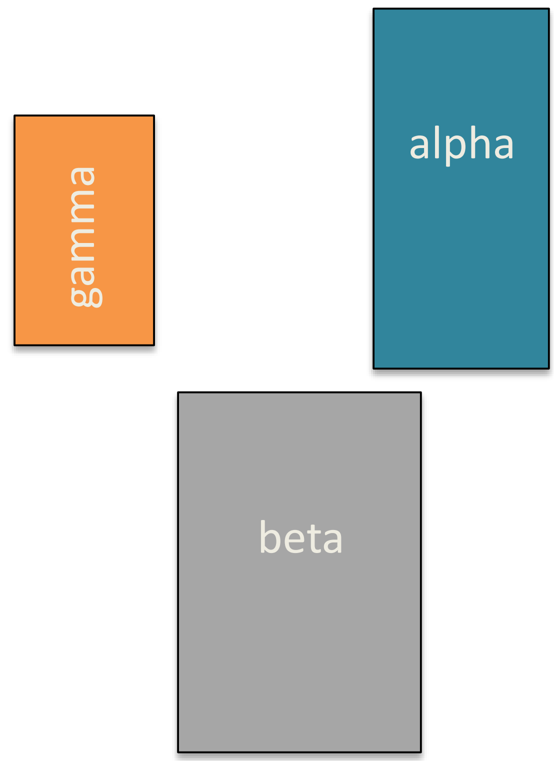
Proper assembly of MMOH is challenging

Assembled Complex



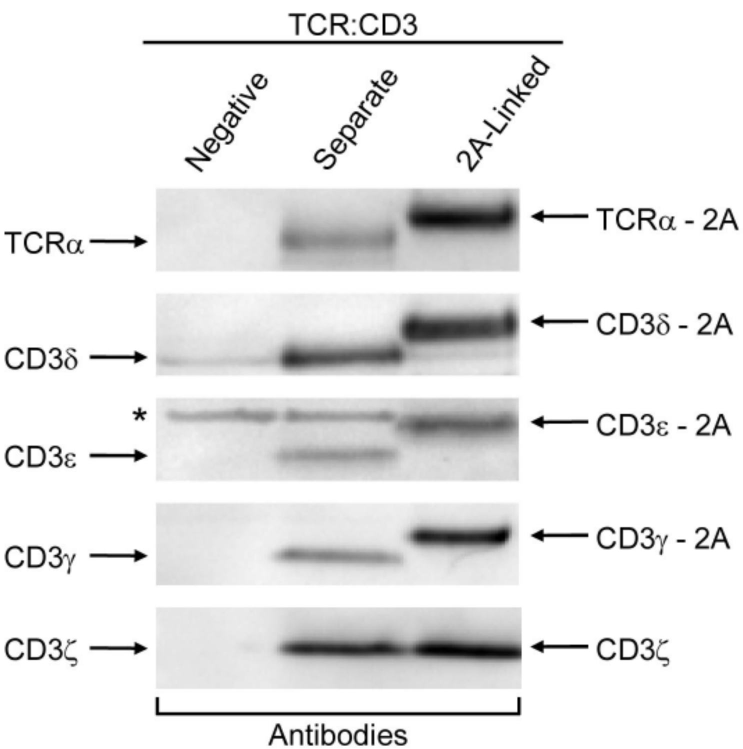
Stable. Active complexes are readily purified from *M. capsulatus*.

Pre-assembled Components



Unknown stability in *Pichia*. Assumed to be unstable.

Type 2a peptides balance component stoichiometry



Szymczak AL *et al*. *Nat Biotechnol* 2004; **22**: 589–594; Epub 2004, April 4.

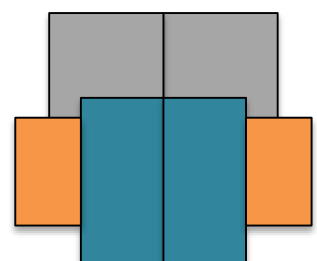
VKQTLNFCLLKLAGDVESNPGP

Ribosome skips this peptide bond

MMOH

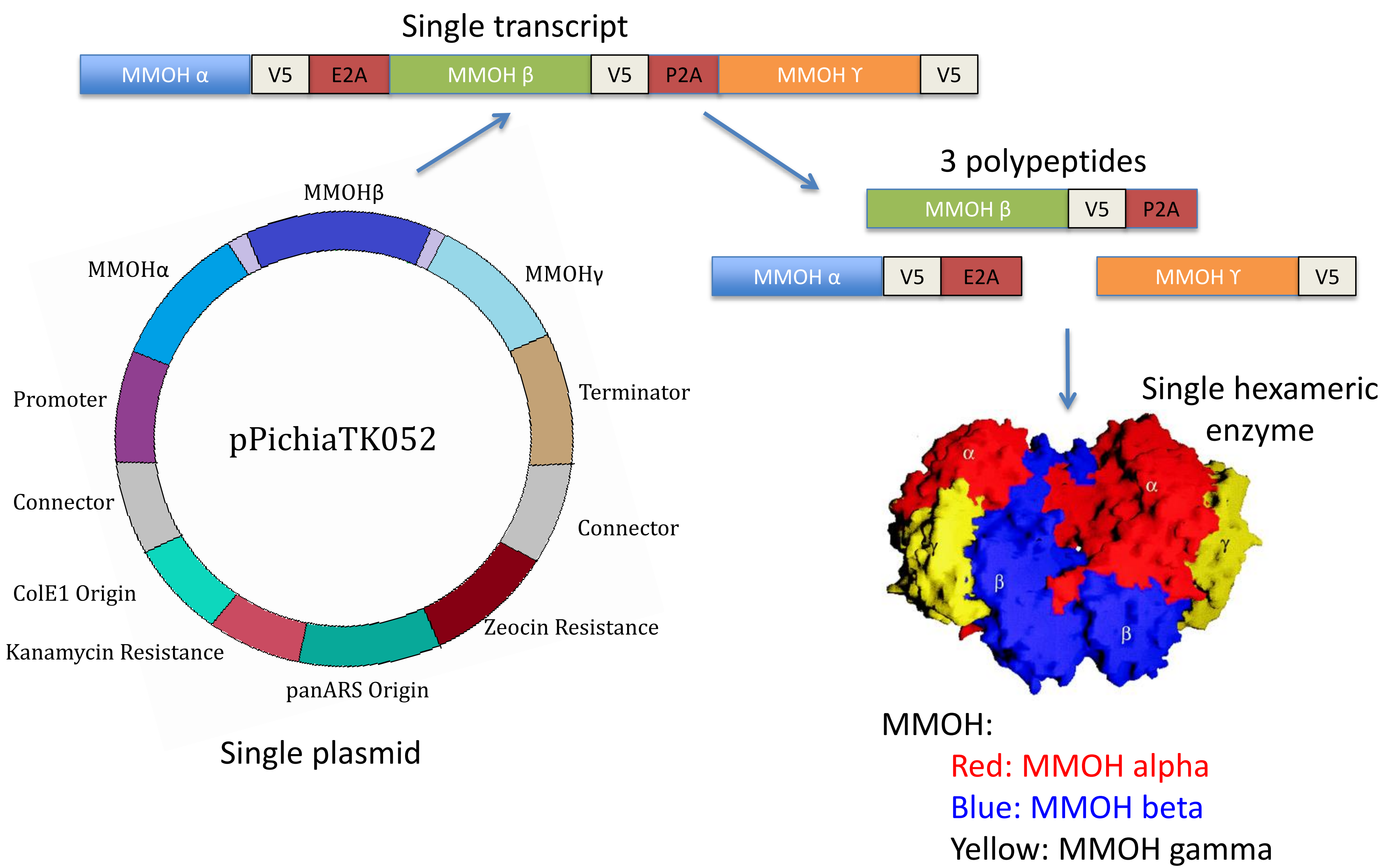
Alpha---2a---Beta---2a---Gamma

Alpha-2a Beta-2a Gamma

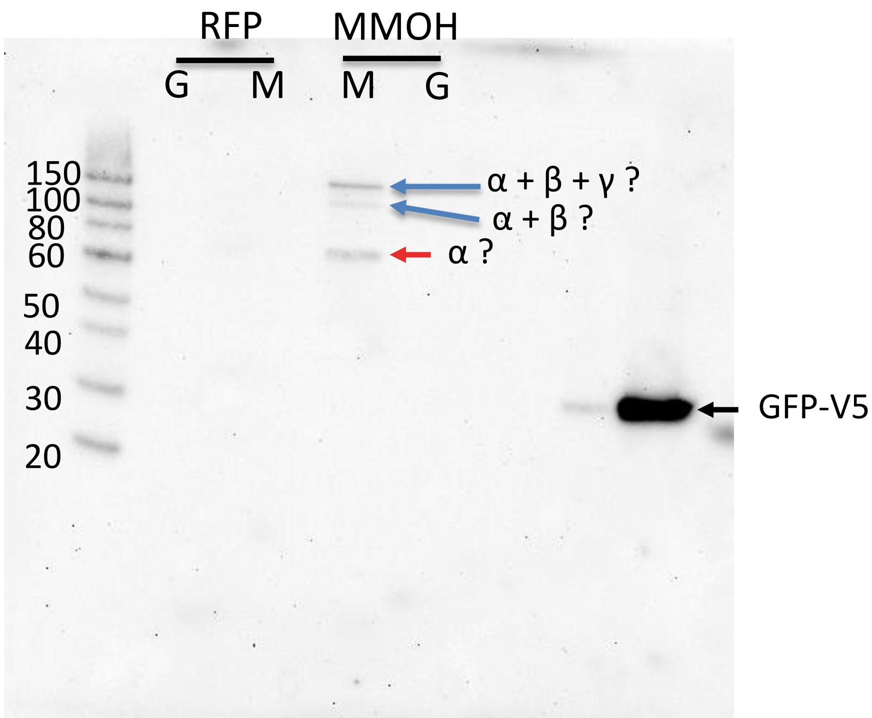


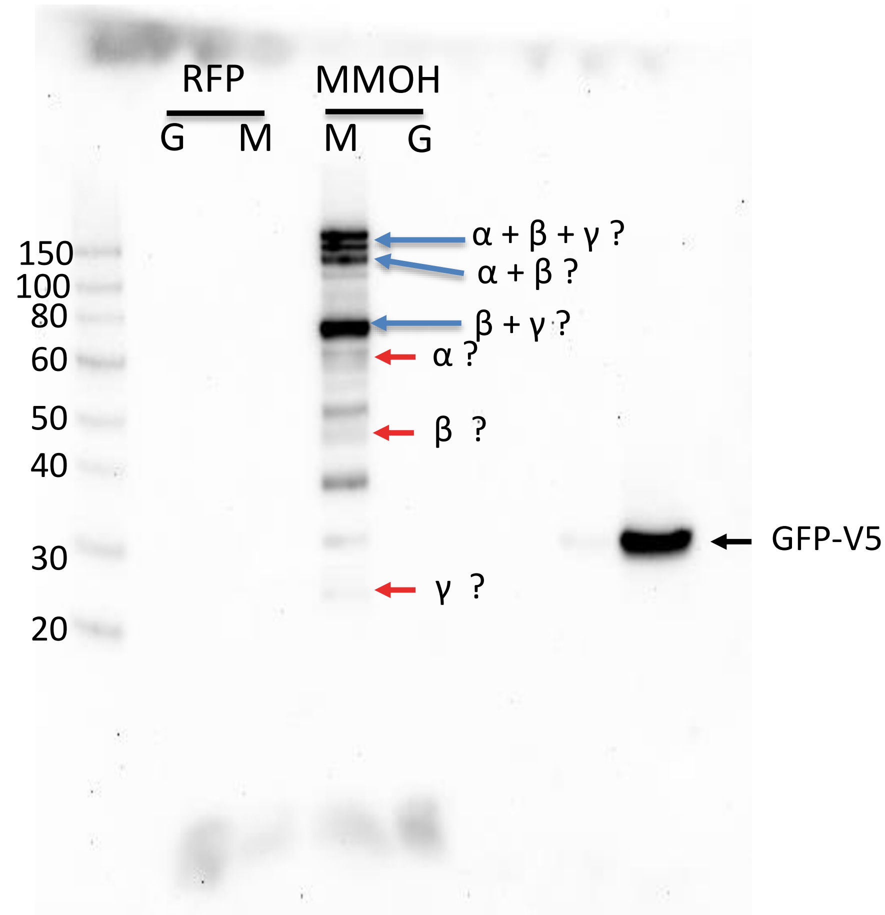
Stoichiometric production of complex components

Expressing MMOH from single transcript



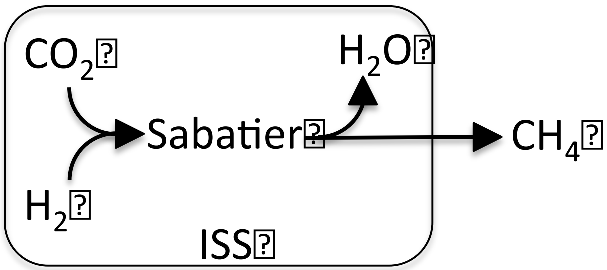
Western blot to detect MMOH subunits

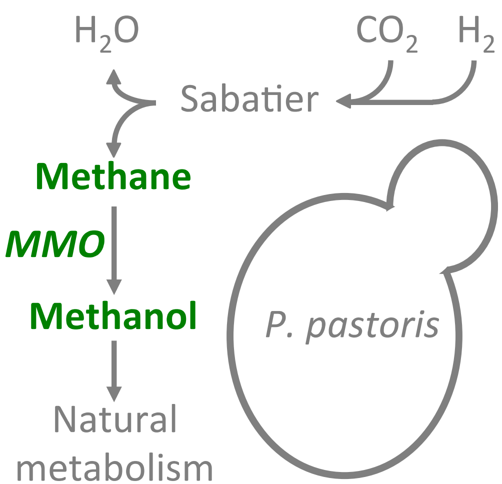




Protein	MW (kDa)
MMOH α	63.5
MMOH β	48.4
MMOH γ	21
MMOH α + β + γ	132.9
MMOH α + β	111.9
MMOH β + γ	69.4

Porting sMMO to *Pichia*: Current status

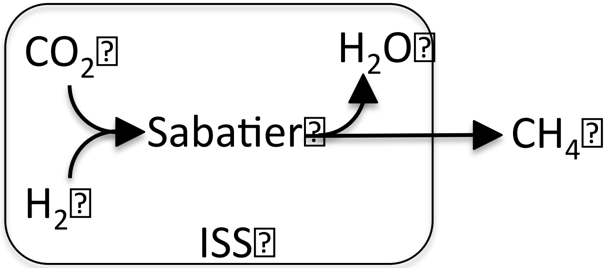


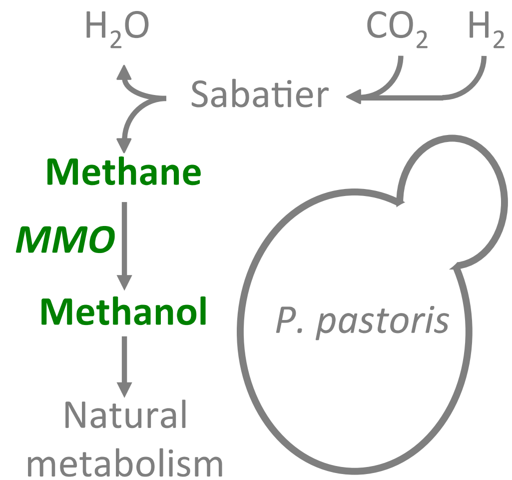


Status

	Designed	Built	Tested
MMOH			*n.a.*
MMOB			*n.a.*
MMOR			*n.a.*
MMOG			*n.a.*
MMOH plasmid			
MMOR/B/G plasmid			

Potential of Biotechnology in Space





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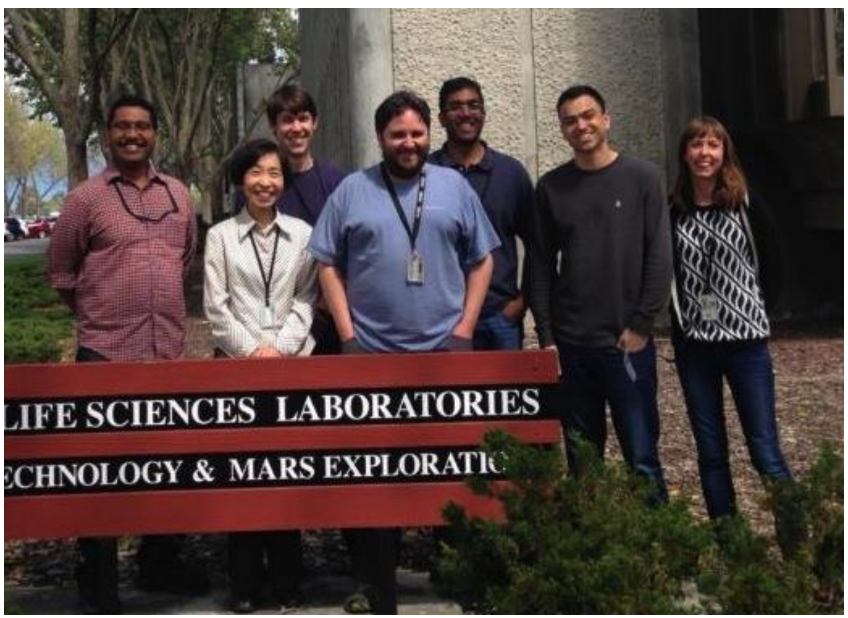

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